Vol. 220 No. 6 DECEMBER, 1950 No. 945

THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

UNIVERSITY OF MICHIGAN
DEC 2 1950
MEDICAL LIBRARY

REG. U. S. PAT. OFF.
ESTABLISHED 1820

Editor: E. B. KRUMBHAAR, M.D.

Associate Editors: RICHARD A. KERN, M.D. WILLIAM C. STADIE, M.D.

W. Lloyd Aycock, Carl Bachman, Herman Beerman, Wm. L. Benedict, Frank B. Block, George E. Burch, Stuart K. Bush, G. Lyman Duff, H. P. Wagener, Franklin G. Ebaugh, Cary Eggleston, Noah D. Fabricant, Harry Gold, Irving J. Wolman, John E. Gordon, David M. Gould, N. R. Ingraham, Jr., C. G. Johnston, R. H. Morgan, E. G. D. Murray, I. S. Ravdin, John H. Stokes

A Study of Serum Fat Globules in Atherosclerotic and Non-Atherosclerotic Male Subjects. By Willard J. Zinn, M.D., and George C. Griffith, M.D. 597

Oral Treatment of Pernicious Anemia with Vitamin B_{12}. By Leo M. Meyer, M.D., Arthur Sawitsky, M.D., Bernard S. Cohen, M.D., Mendel Krim, M.D., and Robert Fadem, M.D. 604

Primary Amyloidosis. A Clinical and Pathological Study. By William H. Higgins, M.D., and William H. Higgins, Jr., M.D. 610

Non-Specific Granulomatous Inflammation of the Stomach and Duodenum. By Manfred W. Comfort, M.D., Harry M. Weber, M.D., Archie H. Baggenstoss, M.D., and William F. Kiely, M.D. 616

Meningitis Due to Pseudomonas Aeruginosa Treated with Polymyxin B. By E. R. Hayes, M.D., and Ellard Yow, M.D. 633

Cultural Properties and Pathogenicity of Certain Microörganisms Obtained from Various Proliferative and Neoplastic Diseases. By Virginia Wuerthele-Casfé, M.D., Eleanor Alexander-Jackson, Ph.D., John A. Anderson, Ph.D., James Hillier, Ph.D., Roy M. Allen, D.Sc., and Lawrence W. Smith, M.D. 638

The Locus of the Peripheral Vasodilator Action of Ethyl Alcohol, Tetraethylammonium and Priscoline. By Lawrence L. Siems, M.D., and H. S. Rottenstein, M.D. 649

The Occurrence of Renal Insufficiency in Subacute Bacterial Endocarditis. By Herman Villarreal, M.D., and Leon Sokoloff, M.D. 655

Studies of Immunological Reactions of Patients with Hodgkin's Disease. By Antonio Rottino, M.D., and George T. Hoffmann, M.D. 662

Inhibition of Hyaluronidase, Using Fluorescein as an Indicator. By Charles R. Shuman, M.D. 665

Experiences with the Dilute Prothrombin Time in the Diagnosis of Thrombo-Embolic Disease. By Julius J. Sachs, M.D. 674

The Diagnostic Significance of Urinary Pepsinogen Excretion in Diseases of the Upper Gastrointestinal Tract. By Henry D. Janowitz, M.D., Milton H. Levy, M.D., and Franklin Hollander, Ph.D. 679

Progress of Medical Science

Gynecology and Obstetrics. Carl Bachman, M.D., and Frank B. Block, M.D. 683

Pediatrics. Irving J. Wolman, M.D. 697

Proceedings of the Physiological Society of Philadelphia 707

Complete contents on adv. page 10

OWNERS AND PUBLISHERS LEA & FEBIGER PHILADELPHIA 6, PENNA.

COPYRIGHT, 1950, BY LEA & FEBIGER. ALL RIGHTS RESERVED. ENTERED AS SECOND-CLASS MATTER NOVEMBER 5, 1887 AT THE POST OFFICE AT PHILADELPHIA, PA. UNDER THE ACT OF MARCH 3, 1879

PUBLISHED MONTHLY. SUBSCRIPTIONS, TEN DOLLARS A YEAR

GRADUAL

PROLONGED

SAFE

PROVE it yourself with HEPVISC. The ideal Vasodilator in the treatment of vascular hypertention.

Clinical studies and pharmacological tests of HEPVISC indicate:

GRADUAL REDUCTION
PROLONGED ACTION
THERAPEUTIC SAFETY

HEPVISC is a synergestic combination of hexanitrate of mannitol and viscum album extract.

An ideal therapeutic product in reducing blood pressure.

COMPOSITION

Each tablet contains 8 mg. hexanitrate of mannitol and 50 mg. solid extract viscum album.

DOSAGE

2 tablets three or four times daily as needed.

HEPVISC

Reg. U. S Pat. Off.

Hexanitrate of Mannitol plus Viscum Album Extract

Supplied in bottles of 50-500-1000 tablets

Available at All Drug Stores

Samples and complete literature sent to physicians on request

ANGLO-FRENCH LABORATORIES, INC.

75 VARICK STREET, NEW YORK 13, N. Y.

THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

DECEMBER, 1950

ORIGINAL ARTICLES

A STUDY OF SERUM FAT GLOBULES IN ATHEROSCLEROTIC AND NON-ATHEROSCLEROTIC MALE SUBJECTS

By Willard J. Zinn, M.D.*

AND

George C. Griffith, M.D.

LOS ANGELES, CALIFORNIA

(From the University of Southern California, School of Medicine, Department of Cardiology, and the Los Angeles County Hospital)

Since James Edmunds[1] in 1877 reported bright motes in blood as seen with the use of a paraboloid condenser, sporadic attempts have been made by numerous investigators to identify and make use of these glittering particles. In 1924, Gage and Fish[8] in this country published their findings relating these particles to the transport of neutral fat. They named the large (½ to 1 micron) globules "chylomicrons". By chemical, physical, and physiological study procedures they felt that they had offered ample proof that these chylomicrons were neutral fat particles.

Between 1936 and 1942, Frazer and his co-workers[2,3,7] in England re-evaluated both the composition and the role of these particles seen in serum by dark field illumination. Once again it was concluded that they were neutral fat particles with a probable globulin coat. More recently, Moreton[13,14], of Salt Lake City, revived interest in these particles, feeling that they could be related to Hueper's[11] theory of the pathogenesis of atherosclerosis. Not only will the large size of these particles fit his macromolecular coat hypothesis, but particles of this size might be treated as foreign bodies which have experimentally produced atherosclerosis in the hands of such men as Hirsch and Weinhouse[10], Pollak[15], and Leary[12].

The most recent and definitive work on this problem has been done by Gofman *et al.*[9] at the University of California. They analyzed the cholesterol content of lipoid particles separated by ultracentrifugation. Larger than normal particles were implicated in their work, but chylomicrons were too variable to be of significance.

Using Frazer's[7] terminology and a variation of the simplest procedure described by Moreton[13,14], a qualitative study of lipomicrons (any fat particle) and chylomicrons (large fat particles) was begun.

* Trainee of the National Heart Institute at Bethesda, Maryland.

Method. The apparatus consisted of a Spencer microscope with dark-field substage and lens. The magnification was approximately 1800 x. A 20 x ocular was used to cut down the field of vision and a net micrometer was inserted. Samples of capillary blood were collected by finger puncture in 1 mm. bore capillary tubes. Duplicate samples were drawn for each determination. The ends of the tubes were sealed. They were centrifuged sufficiently to separate serum from clot. The serum was transferred to a glass slide; cover slip was affixed, and the count made.

The counting procedure itself was done by estimation of the total number of particles and the number of grossly large particles seen in a single large square of the net micrometer at 1 level of focus. The field was shifted and the count repeated in 50 locations, if few particles were present, and 10 locations if there were a large number of particles per square. One hundred squares approximated 1 dark field.

All samples were unknown to the investigator at the time the counts were made. Identification labels were removed and substitute symbols added with a master chart kept by a person other than the microscopist.

Fasting samples were insured by placing the patients on a diet that provided no fat in the evening meal. Blood was then collected before breakfast the following morning. Most of the patients were given a breakfast containing 20 gm. of fat. To insure a normal absorption of this amount of fat, it consisted of 2 eggs, 2 pats of butter, toast, porridge without cream or milk, and black coffee in a calculated amount to provide the 20 gm. of fat. Duplicate samples were then taken at hourly intervals for 5 or 6 hours after the fat meal. The fasting sample was repeated on 4 separate days. The discomfort and annoyance of missing lunch and eating an unpalatable fat-free supper for 4 days limited the number of volunteers for this study.

EVALUATION OF THE EXPERIMENTAL TECHNIQUE. Even for qualitative purposes, the multiple sampling errors encountered from the time of the collection of the blood to the actual count appeared to be large. To determine the experimental error of the procedure, patients had 8 samples drawn at one time, which were separately centrifuged and counted. In the absorptive period, 10 separate squares were counted in 10 different fields on each sample. Tabulation of the results for both lipomicron and chylomicron counts showed a variation of from 139 to 184 total particles to 32 to 50 large particles. The percentage of the total particles found to be large for each sample varied from 25 to 31. The coefficient of variation was 12% for the percentage of the total particles that were large in each sample.

In the fasting state the variation was wide in both total particles counted and large particles counted, and the proportion of large to total, as studied in 9 patients, showed coefficients of variation ranging from 5 to 29%. These samples were taken on 4 separate days and gave some indication, therefore, of the day-to-day variation to be expected.

Bearing the wide limits of technical error well in mind, the procedure was applied as previously described, to the study of some 23 patients who had had old myocardial infarctions, 15 patients with no clinical evidence of atherosclerosis, and a random sample of 10 diabetic ward patients.

Results. *Non-atherosclerotic Patients.* For a patient to fall clinically into this group, it was necessary that he have no history of myocardial disease, stroke, or evidence of peripheral arteriosclerosis. It was assumed that any lipoid disturbance associated with clinical atherosclerosis might manifest itself in blood years before the appearance of signs or symptoms. With this assumption in mind, control patients were preferably over 50 years of age. In this way, it was felt that they had adequately demonstrated their ability to resist atherosclerotic disease over the major portion of the normal life span.

The fasting blood of 15 such patients was studied. In 6 patients, 20 gm. fat test meals were administered and the blood checked hourly for 5 hours. The crosshatched columns in

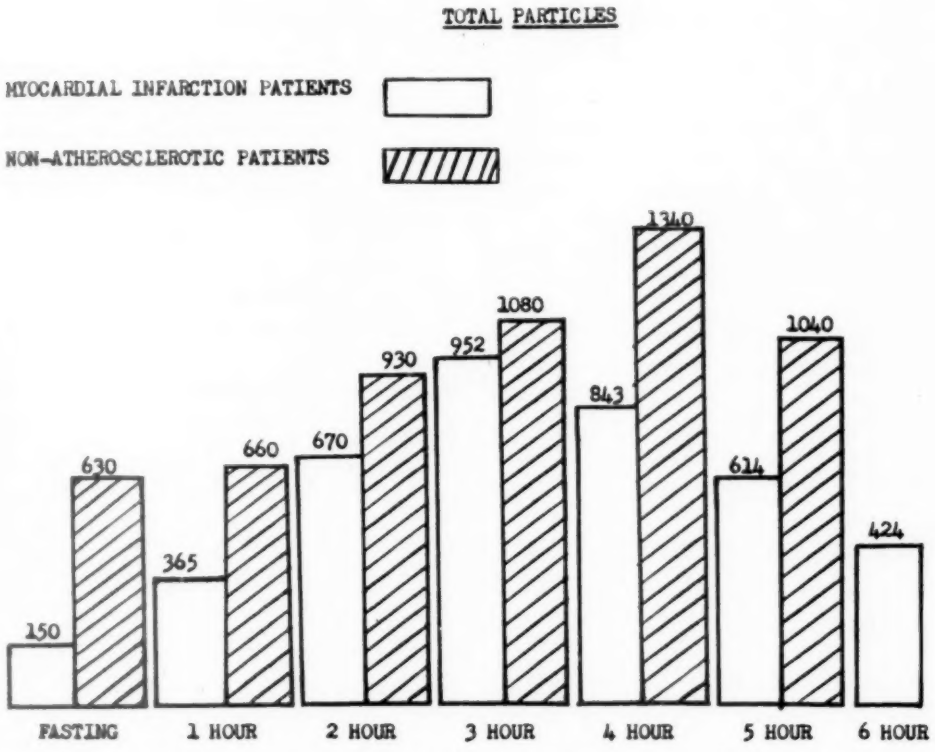


FIG. 1.—Average findings in patients subjected to 20 gm. fat meal tolerance test.

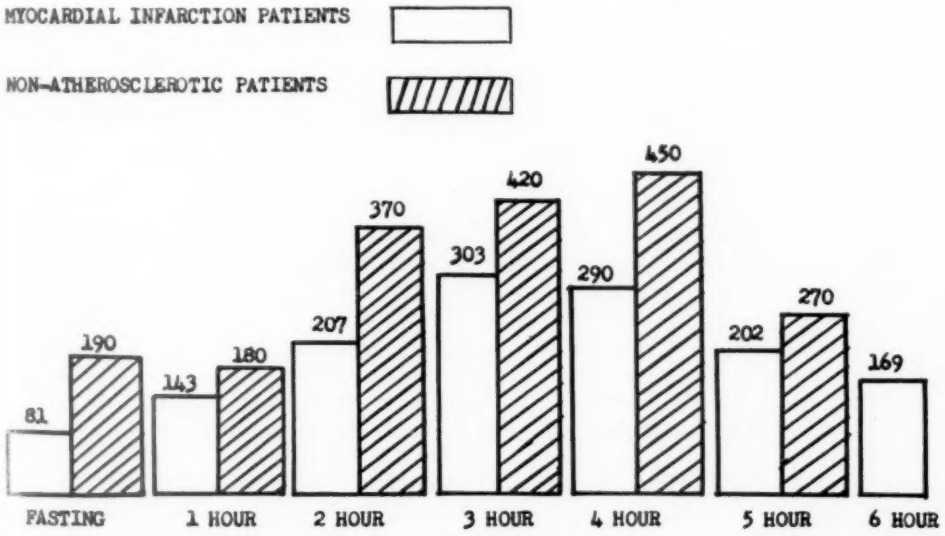


FIG. 2.—Average findings in patients subjected to 20 gm. fat meal tolerance test.

Figs. 1 to 3 represent the average findings in the patients subjected to the test meal. On preparation of these tables, it was seen that the only consistent, striking variation between the atherosclerotic and the non-atherosclerotic group was found in the proportion of large to total particles in the fasting blood. Throughout the rest of this paper, this ratio will be expressed as the percent of the total number of particles found to be large. 5 years before study was accepted as clinical evidence of atherosclerosis. Twenty-three patients were studied for lipomicron and chylomicron levels in a fasting state. They were given the 20 gm. fat meal and followed for 6 hours thereafter. The unlined columns in Figs. 1 to 3 represent the average results of the study. The ratio of large chylomicrons to total lipomicrons expressed as a percent of the whole was found to average 55% among these 23

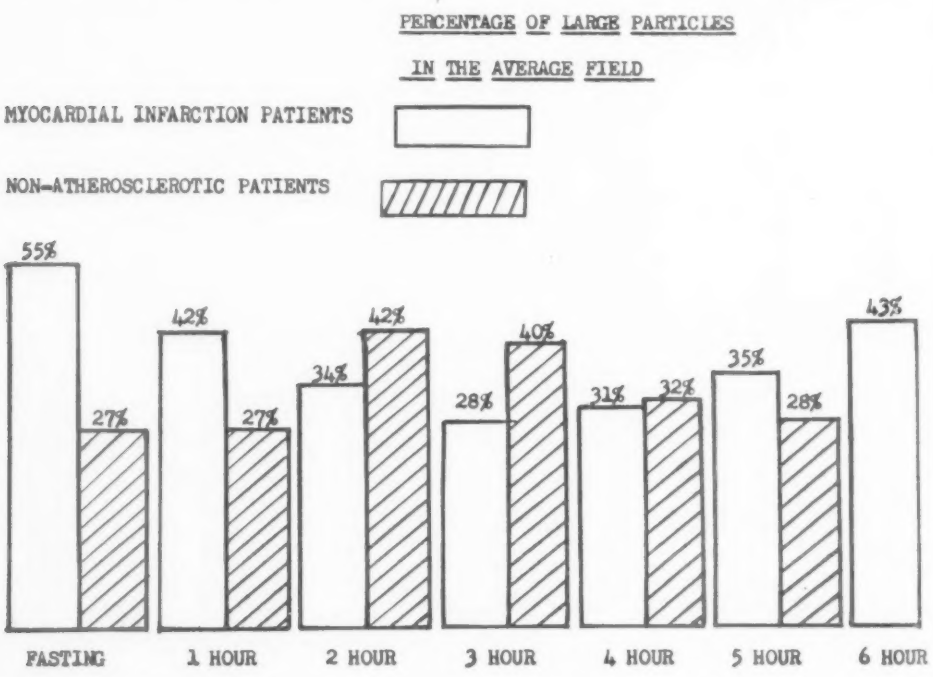


FIG. 3.—Average findings in patients subjected to 20 gm. fat meal tolerance test.

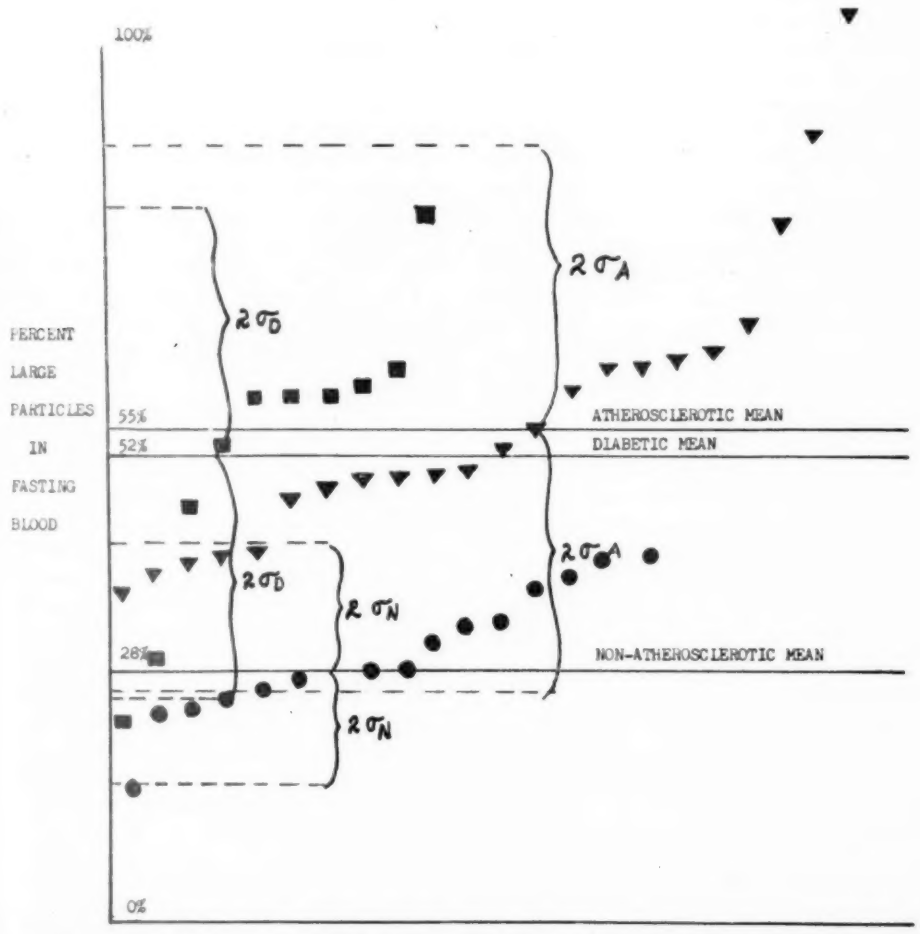
The mean value for the 15 control subjects was 28%. The standard deviation was 6.5%. The control group represented not only persons in normal health, but patients with lung abscess, cirrhosis of the liver, cancer of the rectum, lung, and sigmoid colon, asthma, rheumatoid arthritis, and traumatic fractures.

Atherosclerotic Group. The proven existence of 1 or more myocardial infarctions occurring from 3 weeks to subjects. The standard deviation in this group was 15.6%.

Diabetic Group. Knowing the recognized clinical relationship between early atherosclerosis and diabetes mellitus, it was felt that a random sample of patients on a diabetic ward would be of interest. Fasting blood was drawn from 10 unselected ward patients. The results expressed as the percent of chylomicrons in the total lipomicron count show an average of

52% with a standard deviation of 14.7%. From Fig. 4 it will be seen that 2 of these patients fell well within the limits of the control subjects, and were widely separated from the average values of the other diabetic patients.

Discussion. Calculation of the standard error of the difference between the means of the control and the atherosclerotic group shows it to be 3.7%. The difference between the 2 means is 27% which is greater than 7 times the standard error. This is well beyond a chance variation. The standard error of the difference between the diabetic and atherosclerotic group means is 5.4%. Since the difference between the means is 3%, there is no significant difference between the 2 series. From Figs. 1 and 2 it can be seen that both chylomicrons and lipomicrons of both atherosclerotic and non-atherosclerotic patients are similar in absolute figures. Actually, the control group had larger total numbers of both chylomicrons and lipomicrons. In contrast, Fig. 3 shows an entirely different curve for control and atherosclerotic patients following the test meal when only the proportion of



IG. 4.—Individual patients. The circles represent the non-atherosclerotic patients. The squares represent the diabetic patients. The triangles represent the atherosclerotic patients.

large particles per average field is represented. This result is caused by the marked increase in the proportion of chylomicrons to total lipomicrons in the fasting samples from atherosclerotic patients. This increase is not noted in the control subjects. During the absorptive period, not only do both lipomicrons and chylomicrons rise, but the rise of small lipomicrons is sufficiently great in the atherosclerotic subjects to reduce the proportion of chylomicrons to approximately control values. This would indicate the possibility that these tests show that atherosclerotic patients absorb and transport fat in much the same way as do the control patients. The increasing discrepancies at either end of these curves suggest that the main difference here shown in the atherosclerotic patient is one associated with the transport of metabolized fat, and not primarily with initially absorbed fats.

Further consideration of the fasting proportions of chylomicrons in control and atherosclerotic patients leads to some interesting speculations on the validity of this procedure in the early recognition of atherosclerosis. Working from distribution tables it was found that there was approximately 1 chance in 20 of an atherosclerotic patient similar to those studied in this series having a value equal to the mean value of the control subjects. On the other hand, there was less than 1 chance in 1000 that a patient having the mean value for the myocardial infarction group would be found among non-atherosclerotic patients.

Summary. A qualitative study has been undertaken to use the dark field microscopic technique for visualization of neutral fat globules. This has been carried out on blood sera of non-atherosclerotic and atherosclerotic male subjects. Control subjects consisted of both normal patients and patients with illnesses unrelated to atherosclerotic disease. Myocardial infarction patients were used to represent the atherosclerotic group. A random sample of 10 hospitalized diabetic patients was included.

It was found that there was a significantly greater ratio of chylomicrons to lipomicrons in the fasting samples of atherosclerotic patients. This could not be observed during the period of peak absorption following a 20 gm. fat test meal. The significance of these findings was discussed.

Conclusions. 1. There is a significant qualitative difference in the physical size of the lipid particles observed under dark field illumination of the sera of atherosclerotic and non-atherosclerotic patients.

2. Cancer of the rectum, cancer of the sigmoid colon, cancer of the lung, lung abscess, asthma, rheumatoid arthritis, cirrhosis of the liver, and traumatic fractures do not influence this variation as seen in the patients studied in this series.

3. Diabetic patients sampled from hospital admissions are similar to the atherosclerotic group represented by myocardial infarction patients.

4. The similarity of the absorption of fat by the 2 groups with respect to actual lipomicrons and chylomicrons determined, and the wide discrepancy in the proportion of chylomicrons in the 2 groups in both fasting and late post-absorptive states suggests that the fundamental difference between these 2 groups lies in the metabolic control of fat transport and not in the absorption of the fat across the intestinal mucosa.

ADDENDUM: Since the original preparation of this paper, the number of cases with myocardial infarctions studied has increased to 30, the number of non-atherosclerotic patients to 25, and the number of diabetic patients to 20, without significant change in the mean values, but with a decrease of approximately 1% in the standard deviations.

REFERENCES

1. **Edmunds, J.**: Monthly Microscopy J., **18**, 78, 1877 (cited by Elkes, J. J., *et al.*[2]).
2. **Elkes, J. J., Frazer, A. C.,** and **Stewart, H. C.**: J. Physiol., **95**, 1, 1939.
3. **Frazer, A. C.,** and **Stewart, H. C.**: Ibid, **87**, 53, 1936.
4. Idem: Ibid., **87**, 72, 1936.
5. Idem: Ibid., **89**, 31, 1937.
6. Idem: Ibid., **90**, 18, 1937.
7. **Frazer, A. C.,** and **Walsh, V. G.**: Ibid., **78**, 467, 1933.
8. **Gage, S. H.,** and **Fish, P. A.**: Am. J. Anat., **34**, 1, 1924.
9. **Gofman, J. W., Lindgren, F., Elliott, H., Mantz, W., Hewitt, J., Strisower, B., Herring, V.,** and **Lyon, T. P.**: Science, **111**, 166, 186, 1950.
10. **Hirsch, E. F.,** and **Weinhouse, S.**: Physiol. Rev., **23**, 185, 1943.
11. **Hueper, W. C.**: Arch. Path., **33**, 267, 1942.
12. **Leary, T.**: Ibid., **32**, 507, 1941.
13. **Moreton, J. R.**: Science, **107**, 371, 1948.
14. Idem: Ibid., **106**, 109, 1947.
15. **Pollak, O. J.**: Am. Heart J., **38**, 459, 1949.

ORAL TREATMENT OF PERNICIOUS ANEMIA WITH VITAMIN B_{12} * †

By Leo M. Meyer, M.D.

Arthur Sawitsky, M.D.

Bernard S. Cohen, M.D.

Mendel Krim, M.D.

AND

Robert Fadem, M.D.

NEW YORK, NEW YORK

(From the Third (N.Y.U.) Medical Service, Goldwater Memorial Hospital, New York City, and the Medical Service of the Bronx Veterans Hospital, Bronx)

The efficacy of parenteral administration of vitamin B_{12} in the treatment of pernicious anemia, sprue and nutritional macrocytic anemia has been well established since the initial report by West[6]. Oral administration in patients with pernicious anemia has been accomplished by adding gastric juice from normal individuals or an extract of hog duodenal mucosa to 5 or 10 μgm. of vitamin B_{12}[1,2,3,4]. Spies has reported some success with oral therapy without the addition of "intrinsic factor", and noted an effect by giving 500 μgm. once, 800 μgm. twice, 150 μgm. for 20 and 27 days consecutively, and 450 μgm. for 10 daily doses. He failed to get a response using 30, 50, 100 and 400 μgm. in single doses[5]. It is the purpose of this report to present the data on 7 patients with typical Addisonian anemia in relapse who were treated with the daily oral administration of vitamin B_{12}.‡ All of the cases showed hyperchromic macrocytic anemia, negative Roentgen-ray studies of the gastro-intestinal tract, histamine-fast achlorhydria, and megaloblastic bone marrow. Urinalysis and blood chemistry determinations were normal.

Case Reports. Case 1. M. C., male, aged 69, had a history of several episodes suggestive of minor cerebral vascular accidents. At the time of the present admission there were neurologic signs indicative of involvement of the right internal capsule. The hemoglobin and erythrocytes were 7.0 gm. and 1.60 million per cmm., respectively (Fig. 1). He received single oral doses of 25 and 50 μgm. of vitamin B_{12} without any hematologic or clinical effect. The daily oral administration of 25 μgm. of vitamin B_{12} for 10 days also proved ineffective. Following the daily ingestion of 75 μgm. per day for 12 days, an insignificant rise of reticulocytes to 3.2% occurred on the 8th day. The hemoglobin and erythrocytes increased slightly to 8.0 gm. and 2.04 million per cmm. The dose was then raised to 100 μgm. daily. Although no reticulocytosis was observed, the hemoglobin and red blood cells increased rapidly to 12.0 gm. and 4.03 million per cmm. after 6 weeks of treatment. During this period there was a gradual decrease in symptoms and an improvement in general well being. After the dose was raised to 150 μgm. of vitamin B_{12} daily, the hemoglobin values reached essentially normal levels.

* Presented at the Clinical Research Meeting, New York Academy of Medicine, March 1, 1950.

† Sponsored by the V. A. and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are a result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

‡ Vitamin B_{12} (Rubramin) was supplied by Dr. Geoffrey Rake of the Squibb Institute for Medical Research, New Brunswick, New Jersey.

CASE 2. F. N., female, aged 69, was admitted to the hospital because of weakness and pallor of 3 years' duration. She had a smooth tongue. Neurologic status could not be determined because of the patient's mental confusion. The initial hemoglobin and erythrocytes were 6.5 gm. and 1.75 million per cmm. respectively (Fig. 2.) Following a single oral dose of 25 μgm. of vitamin B_{12} no hematologic or clinical remission was observed. The administration of 50 μgm. once was followed by a slight reticulocytosis of 5.3% and an increase of hemoglobin to 8.0 gm. and red blood cells to 2.35 million per cmm. This dose was then given daily for 8 days but no further response was obtained. When the medication was increased to 75 μgm. per day, no reticulocyte response was observed but the hemoglobin rose to 9.0 gm. and the erythrocytes to 3.5 million. Clinical improvement with mental alertness was apparent. When the dose was raised to 100 μgm. a day the blood rose to hemoglobin 10.8 gm. and 3.80 million per cmm. Another increase to 150, 200, and 300 μgm. per day did not affect the blood values. After the dose was raised to 500 μgm. per day the hemoglobin and erythrocytes rose to 12.5 and

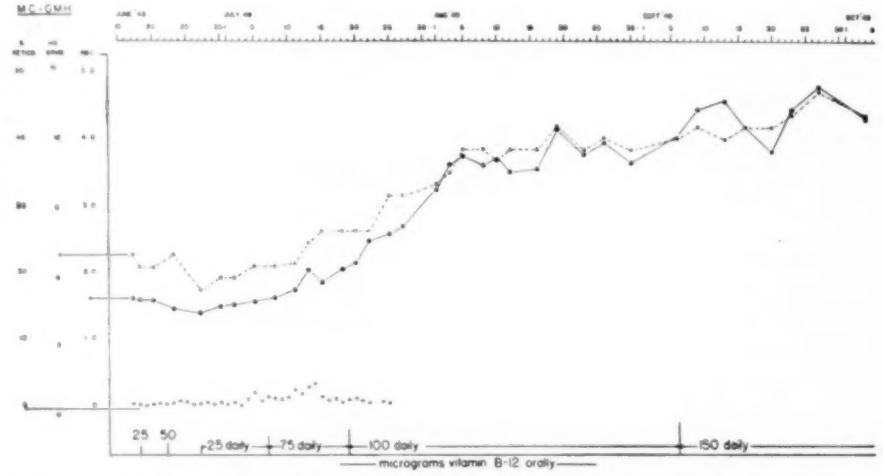


FIG. 1.—M. C. Hematologic data on a patient with pernicious anemia in relapse treated with oral daily doses of vitamin B_{12} varying up to 150 μgm. per day. Clinical and hematologic remission was satisfactory even though the reticulocyte response was poor.

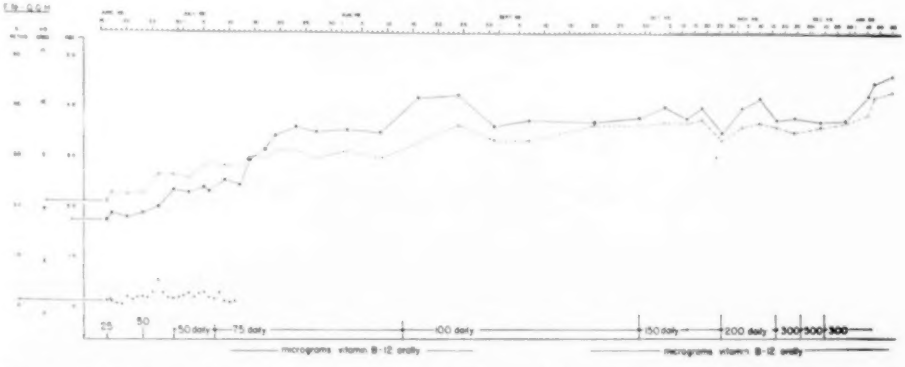


FIG. 2.—F. N. Hematologic data on a patient with pernicious anemia in relapse treated with daily oral doses of vitamin B_{12} varying between 50 and 300 μgm. per day. The clinical and hematologic remission was satisfactory, although the reticulocyte response was suboptimal.

4.50 million per cmm. However, the patient was unable to tolerate the medication and suffered nausea and vomiting. The dose was dropped to 300 μgm. per day and the hemoglobin values have remained normal. The tongue remained smooth but not painful.

Case 3. T. M., male, aged 55, was a known case of bronchiectasis, pulmonary fibrosis, emphysema, and bronchial asthma, with symptoms of active infection. Physical examination revealed signs of bronchopulmonary disease. Neurologic status was normal. The initial blood counts showed hemoglobin 10.5 gm. and red blood cells 2.76 million (Fig. 3). He received single oral doses of 25 and 50 μgm. of vitamin B_{12} with no clinical improvement. The administration of 50 μgm. of vitamin B_{12} daily was equally ineffective. After 100 μgm. per day, a reticulocyte peak of 12.8% was observed on the 9th day of treatment. The hemoglobin and red blood cells which had fallen slightly prior to this period gradually rose to 10.8 gm. and 3.3 million per cmm. The dose was raised to 150 μgm. a day. The maximal blood levels reached were hemoglobin 12.0 gm. and erythrocytes 3.65 million per cmm. These values began to fall, even though the patient was continued on this medication and it was increased to 200 μgm. a day. After the parenteral administration of 20 μgm. of vitamin B_{12} twice weekly, the hemoglobin and red blood cells rose to 15.0 gm. and 5.10 million per cmm. During the entire period of study there was distinct evidence of active pulmonary infection and the patient received continuous antibiotic therapy.

Case 4. H. L., female, aged 77 years, was admitted to the hospital because of weakness, anorexia, and pallor of 4 months' duration. On examination the patient appeared icteric. The liver and spleen were enlarged below the costal borders. The neurologic examination showed evidence of peripheral neuritis. The initial hemoglobin was 4.0 gm. and erythrocytes 1.30 million per cmm. (Fig. 4.) She received daily oral therapy consisting of 75 μgm. of vitamin B_{12} for 12 days. There was no reticulocytosis. The hematologic and clinical conditions remained unchanged. The dose was then increased to 100 μgm. per day. This was followed by a reticulocyte response with a peak of 25.3% on the 8th day of treatment. A slow clinical improvement was noted. Signs of peripheral neuritis disappeared. The hemoglobin and red blood cells gradually increased to 12.0 gm. and 3.90 million respectively after 3 months. The medication was increased to 200 μgm. per day, but no change in the hematologic picture was noted.

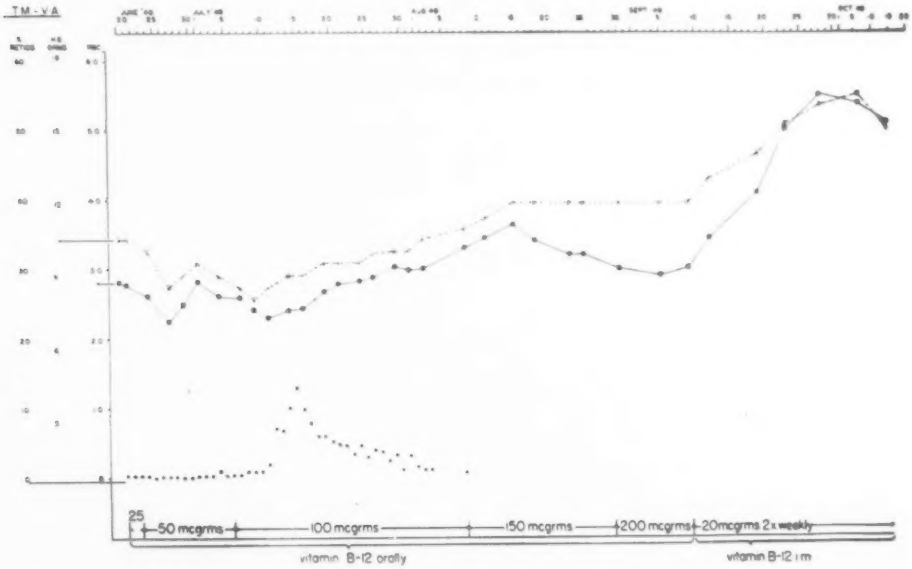


Fig. 3.—T. M. Hematologic data on a patient with pernicious anemia in relapse treated with daily oral doses of vitamin B_{12} ranging from 100 to 200 μgm. per day. The reticulocyte response was satisfactory. A complete hematologic and clinical remission was complicated by an associated bronchiectasis, pulmonary fibrosis, emphysema and bronchial asthma.

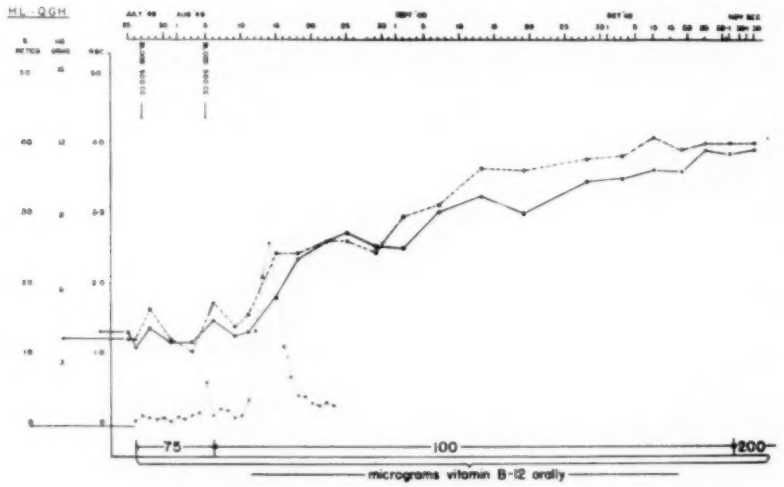


FIG. 4.—H. L. Hematologic data on a patient with pernicious anemia in relapse treated with daily oral doses of vitamin B_{12} ranging from 75 to 200 μgm. per day. The reticulocyte response was satisfactory. The maximal hemoglobin and erythrocyte levels after 3 months were 12.0 gm. and 3.90 million, respectively. Clinical improvement ensued and symptoms of peripheral neuritis disappeared.

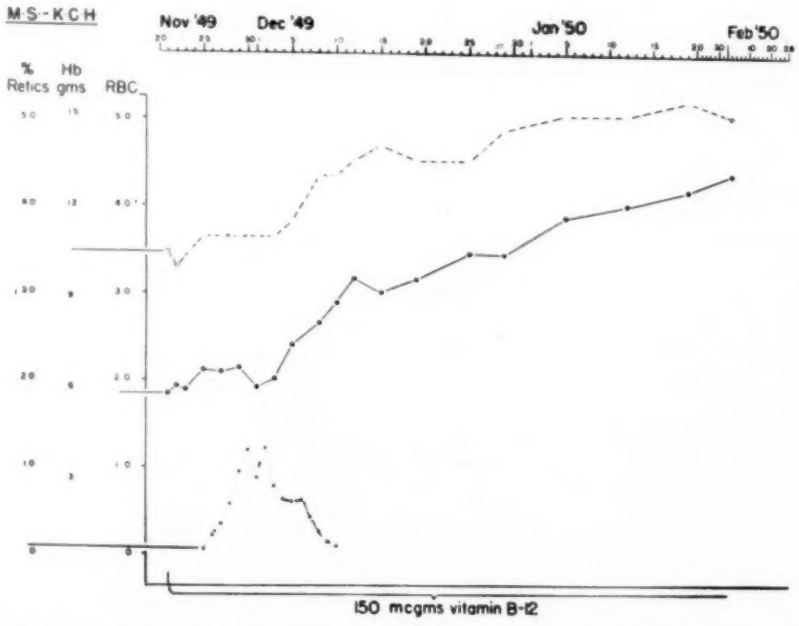


FIG. 5.—M. S. Hematologic data on a patient with pernicious anemia in relapse successfully treated with daily oral doses of 150 μgm. of vitamin B_{12}. Although the reticulocyte response was suboptimal, the rise of hemoglobin and red blood cells was maximal. Clinical remission was complete.

Case 5. M. S., male, aged 46, a known case of pernicious anemia with no treatment for 1 year, was admitted to the hospital because of weakness and pallor. The initial hemoglobin and erythrocytes were 8.5 gm. and 1.85 million per cmm. (Fig. 5). He was treated with daily doses, orally, of 150 μgm. of vitamin B_{12}. Reticulocyte peaks of 12.0% and 12.4% were observed on the 10th and 12th days. Clinical improvement was rapid and the hemoglobin and red blood cells rose to 15.0 gm. and 4.38 million after 10 weeks of treatment. There was no evidence of any neurologic disease.

Case 6. W. C., male, aged 52, was admitted to the hospital because of weakness and pallor of 16 months' duration. Physical examination was negative except for absence of vibratory sense up to the level of the iliac spines. The initial hemoglobin and erythrocytes were 11.0 gm. and 2.79 million per cmm. The patient received daily oral doses of 75, 150, and 250 μgm. of vitamin B_{12} for a total period of 27 days without any evidence of clinical or hematologic remission. Following the intramuscular injection of 30 μgm. of vitamin B_{12} daily for 4 days and 10 μgm. of vitamin B_{12} twice weekly thereafter, there was a return of the blood picture to normal levels in 6 weeks. Although there was symptomatic improvement, the neurologic status remained unchanged.

Case 7. R. F., male, aged 53, was admitted to the hospital because of weakness and parasthesia of the extremities, and recurrent bouts of glossitis, for the past 5 months. Examination disclosed a smooth red tongue. There were signs of posterior and lateral column disease. The initial hemoglobin and erythrocyte levels were 10.0 gm. and 3.22 million per cmm., respectively. He was given oral daily medication consisting of 75 μgm. for 7 days, and 150 for 10 days with no signs of hematologic or clinical improvement. Following the intramuscular administration of 15 μgm. of vitamin B_{12} twice weekly the blood count rose to normal levels in 3 weeks. He was subjectively improved in 6 days and his neurologic complaints were diminished. Neurologic examination, however, disclosed no objective changes.

Discussion. From the foregoing data it appears that there is no clear cut universal oral dose of vitamin B_{12} for the treatment of pernicious anemia. The responses noted would suggest that the deficiency of intrinsic factor is not always complete and that a variable degree of absorption and utilization of vitamin B_{12} (extrinsic factor?) takes place in some cases. It can be assumed that in Cases 6 and 7 no amount of oral therapy would produce a remission without the aid of "intrinsic factor". The persistence of signs of nervous system involvement after parenteral treatment does not mitigate against the value of vitamin B_{12}, as the changes in the cord may be irreversible. Cases 1, 2 and 5 had good hematologic and clinical remissions when given daily oral doses, ranging from 75 to 300 μgm. The vomiting observed with doses of 500 μgm. was probably due to the large number of tablets and amount of water ingested, rather than to the effect of the medication *per se*. The reticulocyte response in this group was absent or sub maximal. Patient 3 showed a satisfactory reticulocytosis, but the hemoglobin and erythrocytes never reached a normal level. Since the patient had an active pulmonary infection a greater need for vitamin B_{12} could only be supplied by parenteral administration. Patient 4 was the only person in the group of 5 who responded to oral therapy who showed evidence of any neurologic disorder prior to treatment. The symptoms of peripheral neuritis disappeared during therapy, even though the hemoglobin and red blood cells did not reach normal levels. The reticulocyte response in this patient was also submaximal. Unfortunately, she has not continued therapy, so that evaluation of parenteral therapy with vitamin B_{12} cannot be determined.

Summary. Seven patients with pernicious anemia in relapse were treated with the daily oral administration of vitamin B_{12} in doses ranging from 75 to 300 μgm. Clinical remission was observed in 5 cases and disappearance of neurologic disease in the 1 patient who had symptoms prior

to treatment. Normal hematologic values were noted in 3 instances and subnormal results were reached in 2 others. In one of these latter cases a severe pulmonary infection was present. This patient had a complete hematologic remission when treated with intramuscular injections of vitamin B_{12}. A maximal reticulocyte response was observed in 1 case, submaximal results in 3 others, and a failure of reticulocytosis was noted in the 5th patient. Two of the patients failed to respond to daily oral doses of 150 and 250 μgm. They subsequently reacted well to parenteral vitamin B_{12} therapy.

REFERENCES

1. **Berk, L., Castle, W. B., Welch, A. D., Heinle, R. W., Anker, R.,** and **Epstein, M.:** New England J. Med., **239**, 911, 1948.

2. **Bethell, F. H., Swendseid, M. E., Meyers, M. C., Neligh, R. B.,** and **Richards, H. G.:** Bull. Univ. Mich. Hosp., **15**, 49, 1949.

3. **Hall, B. E., Morgan, E. H.,** and **Campbell, D. C.:** Prelim. Rept, Proc. Staff Meet. Mayo Clinic, **24**, 99, 1949.

4. **Meyer, L. M., Krim, M.,** and **Sawitsky, A.:** Proc. Soc. Exp. Biol. and Med. In press.

5. **Spies, T., Stone, R. E., Garcia Lopez, G., Milanes, F., Lopez Toca, R.,** and **Aramburu, T.:** Lancet, **2**, 454, 1949.

6. **West, R.:** Science, **107**, 398, 1948.

PRIMARY AMYLOIDOSIS
A CLINICAL AND PATHOLOGICAL STUDY

By William H. Higgins, M.D.

and

William H. Higgins, Jr., M.D.
Richmond, Virginia

(From the Department of Medicine, Medical College of Virgina)

Amyloidosis, first described by Wilks[24] in 1856, is even now an obscure disease still poorly understood and of unknown etiology. It is most commonly encountered in the form of secondary amyloidosis, occurring in such diseases as tuberculosis or chronic suppurative lesions. In this type the amyloid deposits are most abundant in the spleen, kidney, liver and adrenal glands. The earliest deposits occur in and around the walls of capillaries, sinusoids and to a lesser extent small arteries and veins (Eisen[7]). Certain stains are highly characteristic (iodine, Congo Red, and methyl violet).

In contrast there is a rare form known as primary amyloidosis which is thought to differ in the following respects: 1, the deposits occur chiefly in skeletal, cardiac and smooth muscle; 2, they are found only slightly if at all in organs which are the customary sites of secondary amyloid; 3, they tend to be nodular, not diffuse; 4, staining with iodine, Congo Red and the metachromatic dyes yield irregular atypical results (amyloid occurs in more than one slightly different chemical form); 5, associated tuberculous or other causative diseases are lacking.

The classification of amyloidosis is becoming increasingly difficult due to the variability in the organs involved, the clinical symptoms, the atypical tinctorial reactions and inconstancy of a coexisting chronic suppurative process. A suppurative lesion, however, is the chief differential point between the 2 types. In rare instances the deposits are restricted to a single site and occur as tumor like masses often associated with multiple myeloma. The resemblance between the amyloid of multiple myeloma and primary amyloid is striking, as shown by Atkinson[2], in their age incidence and organ incidence, and in their staining properties they are much alike. In myeloma, however, hyperglobulinemia is observed in about 50% of the cases, whereas in cases of primary amyloidosis in which total serum proteins have been determined they have been found to be essentially normal. It has been suggested that when some patients with multiple myeloma develop amyloidosis their serum globulins fall. Several cases are on record bearing out this point[9]. As suggested by Eisen[7] it appears that abnormalities in serum globulins may be related to amyloid formation. Further work along this line is clearly indicated.

Whatever the cause, in amyloidosis a foreign protein, amyloid, is apparently produced and deposited in certain tissues. Although obscure in its exact formation, amyloid (as shown by Hass[12] and others) is composed of 2 protein fractions and 1 polysaccharide. There is evidence that it may be deposited as a combination product, the result of a reaction between a fixed component of the vascular wall and some component of the serum globulin.

Hyperglobulinemia may occur with this reaction and the amyloid may represent tissue deposits of excess globulin protein. A number of years ago Jaffe[14] stressed the relationship between the allergic state acquired during a chronic infection and the appearance of amyloid substance, since secondary amyloidosis is most frequently found in tuberculosis where the occurrence of allergic reactions is so striking. Experimental amyloidosis in mice produced by protein administration has receded when such treatment was terminated. While hypersensitivity to foods or other ingested antigenic materials would suggest an etiologic basis for amyloid deposition, according to Atkinson[2], Rowe has seen no cases of amyloid disease in a large series of allergic patients. In the cases of primary amyloidosis now recorded in the literature (Table 1) an associated allergy was found in only 2 instances. The pathogenesis of amyloid disease is definitely not clearly established. Of the many theories that have been elaborated, the one which relates amyloid formation to hyperglobulinemia has been most favored recently.

In order to formulate a clearer clinical picture of the primary type of amyloidosis this paper concerns itself with a review of the chief clinical manifestations of primary amyloidosis available in the literature and a report of another case entirely symptom free but with sufficient laboratory evidence to place it in this category.

TABLE 1.—INCIDENCE OF LEADING SIGNS AND SYMPTOMS IN 71 CASES OF PRIMARY AMYLOIDOSIS*

Signs and symptoms	*No. cases*	*Per cent*	*New case†*
Congestive heart failure	40	56	0
dyspnea	39	55	0
edema	39	55	0
hydrothorax	18	25	0
ascites	19	29	0
Macroglossia	25	40	Yes
Dysarthria with macroglossia	18	29	0
Dysphagia with macroglossia	15	23	0
General muscular weakness	36	50	0
Deposits in skin and buccal mucosa	25	33	0
Weight loss	28	40	0
Pains in extremities	19	24	0
Abdominal pain	10	12	0
Lymph nodes	18	27	0
Purpura	1	15	0
Epistaxis	3	5	0
Hematemesis	4	4	0
Hematuria	5	7	Yes
Pruritus	5	7	0
Hypertension above (140/90)	13	20	0
Fever	3	5	0
History of allergy	2	3	0

* This table incorporates Eisen's clinical analysis of the first 48 cases up to 1945 with a similar study by the authors of 23 additional cases to date.

† The last column shows the remarkable health of the new case reported in this paper.

As stated, the distinction between the 3 types of amyloidosis is often not clear, but despite this handicap the clinical picture of the primary type is reasonably consistent. In 1946 Eisen[7] summarized the chief clinical data of the recorded cases, bringing the total number in the literature at that time to 48. Since that date the authors have collected 23 additional cases[1,3,4-6,8,10,11,16,22,25] making a total of 71 instances of primary amyloidosis now on record. Errors due to the delay in publication

of the Cumulative Index may cause some omissions, and the confusion arising from the differential diagnosis of the 2 major types add to the difficulty in making an accurate report.

An analysis of the clinical features as previously tabulated by Eisen[7] and supplemented in this paper by the records of the additional cases is shown in Table 1.

In age the patients range from 24 to 90, the average being in the 6th decade. Unlike secondary amyloidosis in which 82% do not survive more than 1 year after the onset of symptoms, the duration of primary amyloidosis is relatively long[21].

The most commonly observed signs and symptoms were related to congestive heart failure. Fifty-three percent in the total published records showed clinical evidence alone of myocardial damage. In this group dyspnea, ankle edema and ascites were commonly found. Lindsay[15] in a study of 43 fatal cases in which autopsies were performed showed that 39 had some degree of amyloid deposition in the heart. In a few cases the amyloid was limited to small cardiac blood vessels. However, in the majority of the recorded cases amyloid was present in the endocardial layer, often as stratified or nodular deposits and occasionally continuous with the myocardial amyloid. The valves were less commonly involved but some showed shotty nodules along the free margins of the mitral and tricuspid valves. Lindsay further brings out the fact that even more important in the mechanism of cardiac failure is the diffuse interstitial amyloid infiltration of the myocardium. Clinical evidence of cardiac failure due to amyloid deposition in the heart may be difficult to evaluate. According to Lindsay[15] the signs and symptoms suggesting cardiac disease may be produced by amyloid involvement: 1, of the lungs with chronic cor pulmonale, 2, of the trachea, or 3, of the mediastinum or by the anemia that often is present. Hypertension is not frequently found.

Lesions of the skin are fairly common. Our records show an incidence of 36%. These manifestations have been described as opalescent and papular[19], nodular[20], sclerodermic[18], eczematous and pink striae[3] between the nails of the fingers and toes.

Macroglossia has been a frequent finding, 40% of the total series showing this localized deposit. In 25% of the cases the enlargement of the tongue was sufficient to produce dysarthria or dysphagia or both. The swelling is usually not painful but superficial ulcerations of the surface have been noted in several instances.

Pains in the extremities and the finger tips are not uncommon, simulating arthritis and producing limitation of motion and disturbances of gait. Enlargement of lymph glands occurred in 27% of the cases resulting occasionally in a localized tumor.

Contrary to an early impression of the infrequency of enlargement of the liver and spleen in primary amyloidosis, 60% of the last 23 cases were reported in the literature as having a clinical involvement of these organs.

More or less complete necropsy reports were available in 54 cases and have been elaborated on by Dahlin[5]. According to his summary, cardiac amyloid infiltration was specifically mentioned in 46 instances and in 25 of these myocardial insufficiency was considered the cause of death. Involvement of the gastrointestinal tract was mentioned in 33 cases. Many of the patients with amyloid in the gastrointestinal canal presented no symptoms referable to this phase of the disease but 5 of them had serious hemorrhage. Pulmonary involvement was noted in 24 cases. Infiltration of amyloid in striated muscle, observed in 19 in-

stances, has been considered the cause for general weakness and limitation of motion commonly complained of. Involvement of the liver and kidneys was present in 21 cases, of the spleen in 20. Amyloid deposits were commonly found in the small arteries and veins.

In view of the accessibility of the amyloid lesions in such a high percentage of cases it is remarkable that in only 14 instances[7,18] in the literature was an antemortem diagnosis made. Biopsies on muscles, tongue, liver, skin, ear, nasal mucosa, and lymph glands gave confirmatory evidences of the underlying lesion in all except 2 where the clinical evidence was conclusive. We include an additional case which was confirmed by liver biopsy.

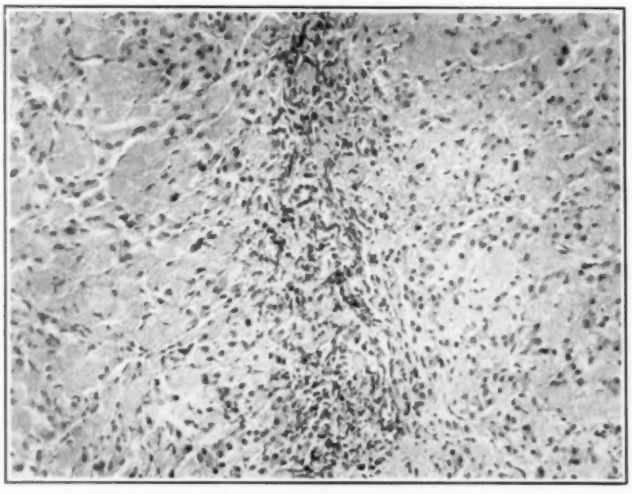


FIG. 1.—Punch biopsy of liver stained with Congo Red. Hepatic parenchyma is so extensively replaced by amyloid tissue that practically no liver cells can be seen.

Case Report. A white 20 year old college student was admitted to the Medical College of Virginia Hospital in March, 1948, because of an enlarged liver. The patient gave an uneventful past history until he was sent to Korea in 1947 for army service when his liver was reported as being enlarged and associated with an albuminuria. He was transferred to Walter Reed Hospital for further observation. His liver was found 3½ finger breadths below the costal border, but his physical findings were otherwise normal. Laboratory examinations established a diagnosis of an acute glomerular nephritis, but the cause of the hepatic enlargement was undetermined. He was discharged from the service 1 year later and has been in excellent health up to the present time. A diagnostic survey of this young man revealed a somewhat underweight individual with no complications. Lungs were clear and cardiac studies including an ECG revealed no abnormalities. Blood pressure was 128/80. The positive findings were a somewhat enlarged tongue, moderately reddened and showing deep indentations. His liver was greatly enlarged, extending below the level of the umbilicus and across the epigastrium to the costal border. The splenic dulness was found at the 7th rib and the edge was palpable. No spider angiomata were found. Cholesterol determinations showed 195 with free 60 and esters 135. Cephalin flocculation was 1+. Total protein was 7.4 with 4.1 albumin and 3.3 globulin. The formal-gel was slightly positive.

Congo Red test on several examinations showed a variation of 75 to 36% retention in the serum. Prothrombin time was 17 seconds, control 15 seconds and concentration was 60. B S P was 12.5 and serum bilirubin was 0 and total was 0.2. Icterus index was 11. Stool examinations for ova were negative. A sternal puncture showed a very slight hyperplasia of the myelocytes with normal platelets and red blood cells. The blood sugar was 72. A

urinalysis revealed a specific gravity of 1.014 with a trace of albumin and was otherwise negative. There was a previous history of hematuria. A chest roentgenogram was normal but showed a splenic shadow as far as the costal border inferiorly and the edge of the liver below the iliac crest. A punch biopsy of the liver was done which was reported by Dr. James Butcher, surgical pathologist at this hospital, as follows (Fig. 1): "The hepatic parenchyma is extensively replaced by homogeneous pink material which specially stained sections give the staining reaction of amyloid. Only small groups of cords of liver cells are preserved and the replacement by amyloid is diffuse throughout most of the sections. Particularly striking is the infiltration of the walls of small blood vessels by this material. The histologic change is regarded as extensive amyloidosis with no suggestion of the underlying etiologic cause." Laboratory studies over a period of 18 months since admission are summarized in Table 2.

TABLE 2.—SUMMARY OF LABORATORY STUDIES OVER 18 MONTH PERIOD

Blood.—Hgb. 12.8 to 13.8 gm.
Rbc. 4.1 to 5.2 million
Wbc. 10,200 to 15,000
Polys 57 to 73%
Lymphs 42 to 25%
Eos. 1 to 2%

Urine.—Sp. Gr. 1.010 to 1.016
Albumin 1 plus
Sugar—Negative
pH—Acid
Micro. 0 to 10–20 Rbc. per h.p.f.

Sternal Marrow.—Slight hyperplasia of myeloid series in myelocyte stage. Platelets and Rbc. normal.

Blood Chemical Findings.—		
Sugar		85 to 100 mg. p. 100 ml.
NPN		31 to 36 mg. p. 100 ml.
Icterus index		7 to 16
Serum bilirubin	Direct	0.0 to 0.2 mg. p. 100 ml.
	Total	0.2 to 0.4 mg. p. 100 ml.
B.S.P.		5 to 12% retained after 5 min.
Thymol turbidity		5 units
Cephalin flocculation		1 plus
Cholesterol	Esters	140 to 160
	Free	20 to 35
Total Proteins		7.4 to 8.6 gm. %
Albumin		4.1 to 3.4 gm. %
Globulin		3.3 to 5.2 gm. %
Formol-gel		Slightly positive
Prothrombin time		17 seconds
Control		15 seconds
Concentration		60%
Congo Red		75 to 36% retention in the serum
Stool exam. for ova		Negative

Comment and Summary. A clinical and pathologic study of primary amyloidosis is presented with a discussion of certain theories having a bearing on the etiologic background of this rare disease.

As far as statistics are available there are now on record 71 cases of primary amyloidosis according to the generally accepted classification. It is undoubtedly true that some cases of primary amyloidosis may simulate the secondary type and it is, therefore, unfortunate that the current criteria of differentiation cannot be more accurate. An illustration of this point is found in the case reported in this paper. This young man has always been in excellent health, a good tennis player, has never shown any evidences of a chronic suppurative process, is free of any proven cardiac involvement and is found in the age group commonly restricted to the secondary type. As noted above, his liver and spleen are definitely enlarged—a finding generally regarded as evidence of this secondary manifestation. On the other hand the absence of a chronic tuberculous or suppurative lesion, the atypical Congo Red stain, the presence

of an early macroglossia seem to afford sufficient proof of a true primary amyloidosis.

Unfortunately studies of the microscopic sections offer little in the differentiation of the 2 types. Until a clearer understanding of the underlying process is brought about, much is left to be desired in clarifying these major subdivisions of this clinical problem.

The extreme rarity of primary amyloidosis as indicated by the statement that up to 1930 only 10 cases had been recorded in the literature is not borne out by studies in recent years. The fact that 48 cases were reported in 1945 by Eisen and that 23 have been added in the last 4 years indicates that there is an actual increased frequency or a keener interest in a problem about which so little is known.

REFERENCES

1. **Arloff, J.**, and **Filder, L.**: Am. J. Med. Sci., **212**, 275, 1946.
2. **Atkinson, F. R. B.**: Med. Press, **195**, 312, 1937.
3. **Baber, M.**: Lancet, **1**, 210, 1947.
4. **Brunsting, A. L.**, and **MacDonald, I. D.**: J. Invest. Dermat., **8**, 145, 1947.
5. **Dahlin, C. D.**: Am. J. Path., **25**, 1, 1949.
6. **Dirkse, R. P.**: Am. J. Roent., **56**, 577, 1946.
7. **Eisen, H. N.**: Am. J. Med., **1**, 144, 1946.
8. **Findley, J. W., Jr.**, and **Wright, A.**: Arch. Int. Med., **81**, 1948.
9. **Freeman, I.**, and **Krause, L. A. M.**: Bull. Sch. Med. Univ. Maryland, **26**, 17, 1941.
10. **Golden, A.**: Arch. Int. Med., **75**, 413, 1945.
11. **Greenbaum, S. S.**, and **Bauer, J.**: Arch. Derm. and Syph., **55**, 251, 1947.
12. **Hass, G.**: Arch. Path., **34**, 92, 1942.
13. **Iverson, L.**, and **Morrison, A. B.**: Ibid., **45**, 1, 1948.
14. **Jaffe, R. H.**: Ibid., **1**, 25, 1926.
15. **Lindsay, S.**: Am. Heart J., **32**, 429, 1946.
16. Idem.: Am. J. Med., **4**, 765, 1948.
17. **Lindsay, S.**, and **Knorp, W. F.**: Arch. Path., **39**, 315, 1945.
18. **Lubarsch, O.**: Arch. f. path. anat., **271**, 867, 1929.
19. **Michelson, H. E.**, and **Lynch, F. W.**: Arch. Derm. and Syph., **39**, 805, 1934.
20. **Mollow, W.**, and **Lebell, H.**: Wien. Arch. f. inn. Med., **22**, 205, 1932.
21. **Pearlman, A. W.**: Quart. Bull. Sea View Hosp., **6**, 295, 1941.
22. **Purcell, M. H.**, and **Brown, E. C.**: U. S. Naval Bull., **46**, 1607, 1946.
23. **Reimann, H. A.**, and **Eklund, C. M.**: Am. J. Med. Sci., **190**, 88, 1935.
24. **Wilks, S.**: Guy's Hosp. Rep., **103**, 11, 1856.
25. **Zemp, E.**: Ann. Int. Med., **26**, 448, 1947.

NONSPECIFIC GRANULOMATOUS INFLAMMATION OF THE STOMACH AND DUODENUM: ITS RELATION TO REGIONAL ENTERITIS*

By Mandred W. Comfort, M.D.
DIVISION OF MEDICINE

Harry M. Weber, M.D.
SECTION ON ROENTGENOLOGY

Archie H. Baggenstoss, M.D.
SECTION ON PATHOLOGIC ANATOMY
MAYO CLINIC

AND

William F. Kiely, M.D.
FELLOW IN MEDICINE, MAYO FOUNDATION
ROCHESTER, MINNESOTA

To Crohn, Ginzburg and Oppenheimer[4] belongs the credit for recognizing, in 1932, nonspecific granulomatous inflammation of the ileum as a clinical entity, although cases had been recorded in previous years[1,8,9]. These authors termed the entity "regional ileitis" but, later, when it was realized that the process involved the jejunum as well as the ileum, the entity received the name "regional enteritis"[2,7].

In recent years we have found that nonspecific granulomatous inflammation may involve the duodenum and the stomach as well as the jejunum, ileum and colon. That this is so does not appear to be well recognized[12], for we have found recorded only 2 cases in which the duodenum was involved[3,11], and 1 in which the stomach presumably was involved[10]. From a larger number we have selected 5 cases of nonspecific granulomatous inflammation in which the stomach and the duodenum were involved, to direct attention to the fact that the disease process does involve this part of the gastrointestinal tract. We will point out the similarity of the pathologic process in the stomach and duodenum to that in the jejunum and ileum and will discuss the manifestations of the disease when the stomach and duodenum are involved.

The term "nonspecific granulomatous inflammation of the stomach and intestinal tract" or "nonspecific granulomatous gastroenteritis", indicating the granulomatous nature of the inflammatory process and involvement of one or more of the divisions of the gastrointestinal tract, appears to be a more suitable name for the disease syndrome than older and less precise names.

Case Reports. Case 1. A man, 26 years old, who registered at the Mayo Clinic in January, 1932, gave a history of episodic, dull, diffuse, upper epigastric pain occurring 20 to 30 minutes after meals and associated occasionally with vomiting. These episodes had been occurring for 3 years. Complete roentgenologic survey of the gastrointestinal tract, cholecystographic examination and routine studies of blood and urine all failed to reveal a significant abnormality. Two hundred cc. of gastric contents were recovered 1 hour

* Read at the meeting of the Minnesota Society of Internal Medicine, Minneapolis, Minnesota, May 21, 1949.

following a test meal. He was dismissed with a diagnosis of indeterminate abdominal distress.

The patient returned to the clinic in April, 1932. After the first admission he had gained weight and had felt well until 1 week previously, when the distress in the abdomen returned. This he localized to the periumbilical area. It was dull, constant and was aggravated by eating. He vomited once or twice daily, the vomitus consisting of food eaten at the previous meal. There was no hematemesis. Roentgenologic examinations of the stomach and colon again failed to reveal evidence of abnormality in these organs. However, roentgenologic examination of the small bowel revealed abnormal motility in the upper coils of the jejunum, associated with slight dilatation. Hemoglobin, erythrocyte and leukocyte values were normal. Fever and sweating did not occur.

Surgical exploration of the abdomen disclosed an extensive hyperplastic jejunitis involving the proximal 6 to 8 feet of jejunum (183 to 244 cm.), the lumen of the bowel being 3 to 4 times larger than normal. The walls of the bowel were thickened and edematous, and the edema extended into the mesentery of the bowel. A moderate amount of straw-colored fluid was present in the peritoneal cavity. The remainder of the small bowel appeared normal to gross inspection. Careful inspection of the peritoneal surfaces did not disclose tubercles. Posterior gastrojejunostomy was performed, using a loop about 8 feet distal to the lowermost area of inflammatory thickening of the jejunum. The patient made an uneventful postoperative recovery and was dismissed 3 weeks after admission.

In September, 1932, the patient returned for examination. He had gained 15 lbs. and was feeling quite well. Roentgenologic examination showed that the gastro-enteric stoma was functioning in a satisfactory manner, but it was not possible to make the contrast suspension enter the 2nd and 3rd portions of the duodenum and these with the orad portion of the long jejunal loop were not visualized. One hour following an Ewald type of test meal, 310 cc. of gastric contents were obtained.

In December, 1932, 2 weeks before readmission, upper midabdominal pain, nausea and vomiting recurred. The distress seemed to be aggravated by meals of more than minimal quantity. Intestinal function was normal.

The patient was hospitalized for treatment and observation. During the ensuing 2 weeks he continued to complain of intermittent periumbilical and epigastric aching pain which often awakened him at night. Vomiting of small amounts of bilestained fluids recurred frequently. A low-grade fever was present intermittently. The abdomen appeared to be distended moderately. A succussion splash was audible. Aspiration of the fasting gastric content consistently obtained from 200 to 600 cc. of residuum. Roentgenologic examination of the stomach and upper part of the small intestine revealed that the gastro-enteric stoma was small but there was no evidence of organic obstruction. The pattern of the mucous membrane of the 2nd and 3rd portions of the duodenum was abnormal but the small bowel caudad to the stoma appeared normal. When the patient was dismissed from the hospital 14 days after admission he had not improved. His condition was diagnosed as recurrent jejunitis and probable similar involvement of the 2nd and 3rd portions of the duodenum.

At the time of his next registration at the clinic, in February, 1933, the patient had gained 22 lbs. Gastric symptoms had disappeared. Roentgenologic examination of the stomach and intestine showed that the pylorus and the gastro-enteric stoma were patent, but the abnormal pattern of the mucous membrane persisted in the 2nd and 3rd portions of the duodenum. Again, the jejunum caudad to the stoma appeared normal. Aspiration of the gastric contents 1 hour following a test meal obtained only 175 cc. of residuum.

In September, 1933, the patient felt well but the abdomen seemed somewhat distended and a succussion splash was quite readily audible over the upper abdomen. Following this visit to the clinic his condition improved and the gastrointestinal disturbance disappeared. Between this and the next examination of the patient, in November, 1947, there was an interval of 15 years. In this period he had served 5 years in the United States Army during World War II without significant disability. His weight at the time of this examination was 206 lbs., and physical examination revealed no abnormalities in the abdomen. His only complaints were referable to chronic prostatitis.

Case 2. A man, 57 years old, first came to the clinic in February, 1948, with a 15-year history of episodic diarrhea. In the early years (1933 to 1943) the diarrheal episodes, occurring every 12 to 18 months, lasted 2 or 3 days and usually were initiated by cramping pain in the left upper part of the abdomen. During the episodes the stools, which

were foul, gaseous and unformed, numbered 6 or 7 a day. About 5 years before his visit to the clinic the episodes of diarrhea began to recur at intervals of approximately 2 months and to last 10 to 12 days. During these periods he passed 12 to 15 stools daily. Cramping pain in the left upper portion of the abdomen, nausea, but rarely vomiting were features of all episodes. Between such episodes he was in the habit of passing one formed stool daily. During the 2nd year before registration he lost about 25 to 30 lbs., but during the year immediately preceding his registration he had been relatively free of such episodes and had gained some weight.

In October, 1947, 4 months preceding admission to the clinic, persistent diarrhea, cramping, bloating, belching and anorexia had recurred; weakness and loss of weight (30 lbs.) followed. Hospitalization had been necessary on 2 occasions.

On admission the patient obviously was ill. Loss of weight and weakness were apparent. Moderate tenderness was noted in the left upper abdominal quadrant. The edge of the liver was palpable 2 fingerbreadths below the right costal border. Borborygmus was audible without the stethoscope. The value for hemoglobin was 13.4 gm. per 100 cc. of blood; erythrocytes numbered 4,970,000 and the leukocytes 9,700 per c.mm. The sedimentation rate was 16 mm. in 1 hour (Westergren method). A stained blood smear revealed only toxic changes in the polymorphonuclear cells. Roentgenologic examination of the stomach and duodenum revealed evidence of hypomotility with an ineffective gastric peristaltic activity and apparently a constriction at the ligament of Treitz (Fig. 1).

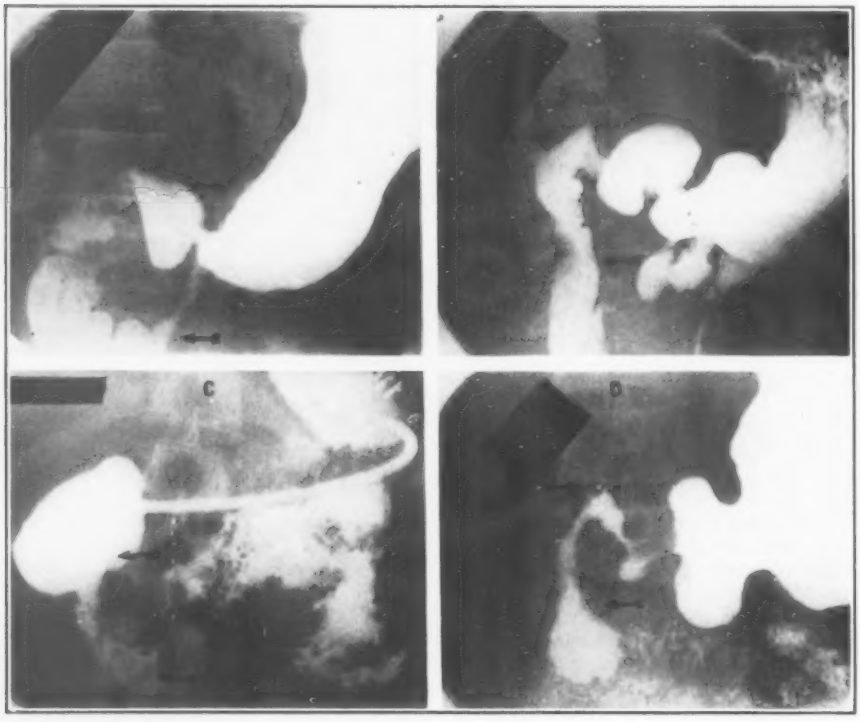


FIG. 1.—The duodenum in Cases 2, 3, 4 and 5. A (Case 2). Tubular constriction of the 2nd and 3rd portions of duodenum (between arrows). B (Case 3). Poorly defined narrowing of the lumen of the 1st (upper arrow) and descending portions of the duodenum (lower arrow). C (Case 4). Irregular tubular deformity of the descending and transverse portions of the duodenum (between arrows). The opaque material was introduced through a duodenal tube. D (Case 5). Irregular tubular narrowing of the lumen of the 1st and 2nd portions of the duodenum (between arrows).

Twenty-four hours later, barium together with considerable retained secretion still was present in the markedly dilated stomach (Fig. 2). Puddling of the suspension of opaque material in the 1st and 3rd portions of the duodenum and slow transit at the duodenojejunal angle were observed. Because of the poor propulsion of barium, these portions of the alimentary tract could not be outlined in a satisfactory manner. There was no roentgenologic evidence of abnormality in the colon and terminal ileum. Proctoscopic examination disclosed a normal-appearing rectal mucosa. Concentrations of serum bilirubin of the indirect-reacting variety, of

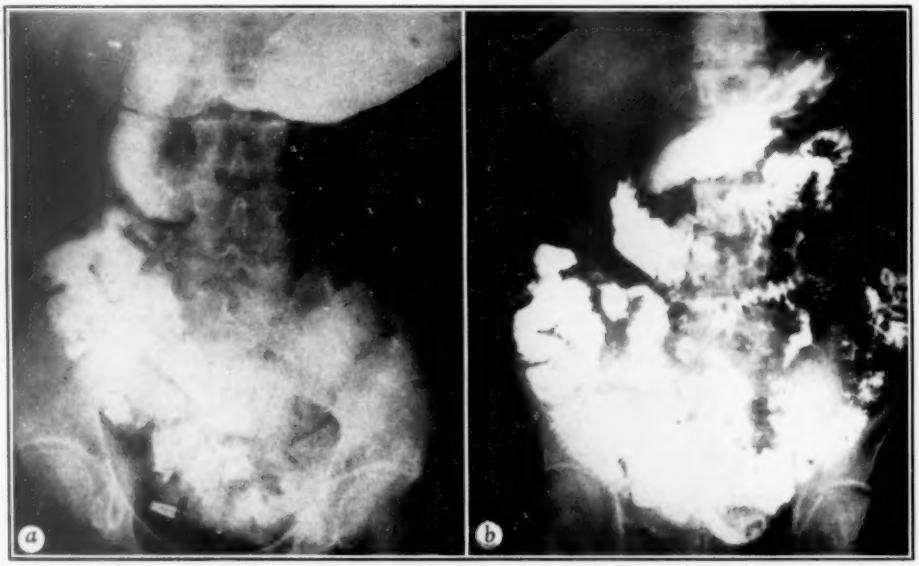


FIG. 2.—Four hours after ingestion of a standard suspension of barium sulfate in water. *a* (Case 2). The stomach, duodenum and jejunum show a moderate degree of dilatation, retention of secretions, and ineffective peristaltic activity. *b* (Case 5). Retarded transit time, "puddling" of the suspension, abnormal secretions and segmentation may be noted. This constitutes the roentgenologic syndrome of the so-called deficiency state.

serum calcium and phosphorus, and of plasma cholesterol and cholesterol esters were, respectively, 0.4, 10.0, 3.0, 87.0 and 64.0 mg. per 100 cc. The concentrations of total protein, albumin and globulin in the serum were, respectively, 6.5, 4.8 and 1.7 gm. per 100 cc. Quick prothrombin time was normal (19 seconds). The sulfobromophthalein test of hepatic function did not disclose retention of dye at the end of 1 hour. Pathogenic ova or parasites were not found on study of specimens of fresh stools. Of the dry weight of a 24-hour stool, 47% was fat. One hour after an Ewald test meal, 275 cc. of gastric contents were aspirated; total acidity was 14 clinical units; free hydrochloric acid was not titrable. Scraps of grapefruit and traces of gross blood were noted in the contents. Free hydrochloric acid was not titrable in the gastric contents obtained fractionally after injection of histamine. On successive mornings, fasting gastric contents measured 250 and 300 cc. and contained fine food scraps and fresh blood. Gastroscopic study revealed diffuse superficial gastritis with multiple areas of superficial ulceration. Clinically, the findings were those of a diffuse lesion of the upper part of the gastrointestinal tract with hypomotility and possible obstruction at the duodenojejunal angle.

Exploratory laparotomy was carried out 3 weeks after the patient's admission. Initial examination of the abdominal and pelvic viscera revealed nothing of significance. Several omental lymph nodes were enlarged. Microscopic study of one of these disclosed an inflammatory process. Because of possible duodenojejunal obstruction, a posterior gastroenterostomy was carried out. On opening the jejunum the surgeon noted that the mucosa lacked the smooth, velvety texture of the

normal mucosa and appeared granular and edematous. Study of fixed and stained sections of tissue from this part of the jejunum revealed moderate jejunitis with edema of the submucosa.

The patient made an uneventful postoperative recovery and was dismissed 3 weeks following his operation, but a letter received in July, 1948, indicated that he was having recurrent episodes of diarrhea similar in every respect to those of which he had complained prior to his visit to the clinic. He had lost a moderate amount of weight and complained of weakness and easy fatigue.

CASE 3. A woman, 31 years old, first came to the clinic in August, 1945. She gave a history of having had in August and in November, 1943, in November, 1944, and in January and in June, 1945, episodes of cramping, periumbilical pain and diarrhea. Each episode of the pain and diarrhea persisted 4 to 5 days. Stools numbered 6 to 12 for each 24 hours and were watery. The fever which accompanied these episodes usually was of low grade but during one of these the temperature reached 103°. Weight was lost rapidly. More recently, the episodes were more severe and of longer duration. In the last episode vomiting occurred. On one occasion the vomitus contained food eaten the day before.

The present episode had been persistent during the 3 months preceding the patient's registration. She had noted carpopedal spasm, occasionally gross blood in the stools, and on one occasion a large crop of subcutaneous hemorrhages. During the past 2 years minimal edema of the feet and ankles, and hypoproteinemia had been noted. Roentgenologic examination had revealed considerable puddling of barium in the proximal duodenum and stomach and a possible obstruction in the 3rd portion of the duodenum.

Her weight was 89 lbs., unclothed; that loss of weight had occurred was obvious. Blood pressure, in mm. of mercury, was 90 systolic and 65 diastolic. Urinalysis gave normal results. The concentration of hemoglobin was 11.9 gm. per 100 cc.; erythrocytes numbered 4,100,000 and leukocytes 8,900 per c.mm. Sedimentation rate was 11 mm. in 1 hour (Westergren method). A stained blood smear revealed generalized macrocytosis but no hyperlobulation of the neutrophilic leukocytes. The blood picture was considered to be not typical of pernicious anemia. Roentgenographic examination of the thorax revealed nothing abnormal. On August 15, 1945, roentgenologic examination of the stomach and duodenum revealed a deformity of the first portion of the duodenum, also a constricting lesion in its 3rd portion (Fig. 1*b*). Three weeks later, roentgenologic examination revealed deformity of the 1st portion of the duodenum but the evidence of constriction in the 3rd portion was not convincing. There was no evidence of abnormality in the colon and terminal ileum. The concentration of serum protein was 5.6 gm. per 100 cc.; the albumin-globulin ratio was roughly 2:1. The concentration of serum calcium was 9.4 mg. per 100 cc. and of phosphorus 3.5. A glucose tolerance test produced a flat type of curve. Culture and examination of the stools revealed no pathogenic organisms or parasites. Examination of a 24-hour collection of feces revealed 41% of the dry weight to be fat, and 5.3 gm. of nitrogen. Gastric analysis 1 hour after an Ewald test meal disclosed 48 units of total acid and 40 units of free acid. The residuum at the end of 1 hour was 40 cc. Proctoscopic examination revealed nothing abnormal.

Exploratory laparotomy was advised and carried out 4 weeks following the patient's registration at the clinic. There was no evidence of duodenal ulcer nor was gross evidence of constriction found in the duodenum or upper part of the jejunum. However, the entire duodenum and upper part of the jejunum were the seat of a mild diffuse inflammatory process. The blood vessels were unusually prominent and small petechiae developed on handling of the bowel. Multiple enlarged lymph nodes were noted in the mesentery. Abnormality of the terminal ileum was not found. The abdomen was closed without further surgical procedures being carried out.

The postoperative course was stormy with considerable upper abdominal pain, repeated vomiting, marked distention of the abdomen and protracted diarrhea. Multiple purpuric spots developed over the trunk and extremities. Six days after operation the prothrombin time, which had been 18 seconds preoperatively, was 46 seconds. Platelet counts, bleeding and coagulation times were normal. A course of Roentgen rays was administered over the abdomen without any immediate improvement in the patient's symptoms, but over a period of 3 months recovery was gradual. The diagnosis was diffuse duodenojejunitis with secondary deficiency state. The patient was dismissed with instructions to use a diet high in calories, carbohydrate and protein but low in fat, with supplementary vitamins. She also was instructed in the intramuscular use of concentrated liver extract.

In March, 1946, the patient wrote that she was feeling better, had gained 11 lbs., but had continued to have episodic diarrhea. In September, 1946, the patient was hospitalized in her native Norway with a recurrence of symptoms, similar to those which she had presented at the clinic. During this course of hospitalization the patient died.

CASE 4. A man, 22 years old, first came to the clinic in April, 1941, because for 3 years he had had episodes of pain, burning in type, located in the left upper quadrant of the abdomen, associated with nausea and vomiting. The vomitus usually contained only that food eaten 1 to 2 hours earlier, but occasionally the morning vomitus contained food particles eaten at supper the evening before. Blood had not been noted in the vomitus. These episodes usually lasted from 1 to 2 days and occurred about once a month, but since January, 1941, they had been more frequent, of longer duration and more severe. The distress had not disturbed his sleep. He had lost 12 lbs. in the preceding 4 months. He was tired and weak. Roentgenologic examination 2 years before admission was reported to show a duodenal ulcer, but ulcer therapy had not relieved his symptoms. In February, 1940, an epigastric hernia had been repaired again without any influence on the recurrent upper abdominal symptoms.

Evidence of moderate loss of weight and pallor were noticeable, but general examination at the clinic otherwise disclosed essentially nothing of significance. Urinalysis gave normal results. The value for hemoglobin was 9.3 gm. per 100 cc. of blood; erythrocytes numbered 5,000,000 and leukocytes 8,500 per c.mm. One hour following an Ewald type of test meal, 850 cc. of gastric contents were removed. The contents contained meat scraps, bile and a trace of blood. The value for total acidity was 20 and free acid was not titrable. Roentgenographic examination of the thorax revealed nothing abnormal but that of the stomach revealed the crater of an ulcer in the duodenum. The stomach was dilated and it contained a fairly large amount of secretion; peristaltic activity of the stomach and duodenum did not appear to be effective. Cholecystographic examination revealed that the gall bladder was functioning in a normal manner.

The diagnosis was gastric retention of unknown etiology. The roentgenologic diagnosis of duodenal ulcer was questioned because of the achlorhydria. The patient was hospitalized for preoperative preparation. He was given a retention type of diet, and the gastric contents were removed by aspiration each morning and night. The large quantities removed daily confirmed the diagnosis of gastric retention.

After 1 week an exploratory laparotomy was carried out at which time the wall of the stomach, pylorus and first portion of the duodenum were markedly enlarged, edematous and thickened. The pyloric ring admitted 2 fingers. Organic obstruction was not present. The diameter of the first portion of the duodenum was approximately 2 to 3 times normal and its wall markedly thickened. In the region of the ligament of Treitz no obstruction was palpable or visible. The jejunum appeared normal but microscopic study of several nodes removed from the mesentery of the proximal portion of the jejunum disclosed an inflammatory process. Exploration of the lower part of the abdominal cavity revealed nothing of significance. The retroperitoneal portion of the duodenum was mobilized, and on inspection a thickened scarred area was noted approximately 2 to 3 cm. distal to the ampulla of Vater. This was believed to be the cause of the obstruction and, accordingly, a posterior gastroenterostomy was carried out. Study of the fixed and stained sections of the gastric wall from the region of the stoma revealed low-grade chronic gastritis. The patient made an uneventful postoperative recovery and was dismissed 3 weeks following operation. The diagnosis was duodenal obstruction due to an inflammatory process of unknown etiology.

The patient was readmitted in April, 1944, 3 years following his first examination. He had gained 10 lbs. and had felt well until about 1 year previously, when he began to experience frequent episodes of diarrhea, fatigue and anorexia and lost all the weight he had regained. Each episode of diarrhea lasted several days. The stools were watery, or soft and unformed, grease floated on the water of the commode and the stools numbered 5 to 6 each 24 hours. Three weeks before admission severe pain developed which was located in the left upper quadrant of the abdomen and extended to the left side of the back. The pain was of great severity for 5 days and was accompanied by nausea, vomiting and fever. The pain and other symptoms persisted in a mild form thereafter.

The patient was apathetic and weak. The blood pressure, in mm. of mercury, was 110 systolic and 80 diastolic. The value for hemoglobin was 7.8 gm. per 100 cc.; erythrocytes numbered 4,580,000 and leukocytes 15,500 per c.mm. The sedimentation rate was 34 mm. in 1 hour (Westergren). A stained blood smear revealed marked hypochromasia with

microcytosis and toxic changes in the polymorphonuclear cells. Aspiration of the fasting stomach yielded 250 cc. of bile-colored residuum containing no free hydrochloric acid. After a histamine stimulus, free hydrochloric acid was not titrable over a 2 hour interval. Roentgenologic examination revealed a somewhat dilated stomach with retained secretion. The stoma of the gastro-enteric anastomosis was patent. The contrast material could not be made to enter the pylorus and duodenum. Forty-eight hours later a Sawyer tube was introduced. After it was ascertained that the tip of the tube was in the duodenum, barium was administered through the tube directly into the duodenum. Roentgenologic examination revealed that the duodenum was dilated above a constricting lesion

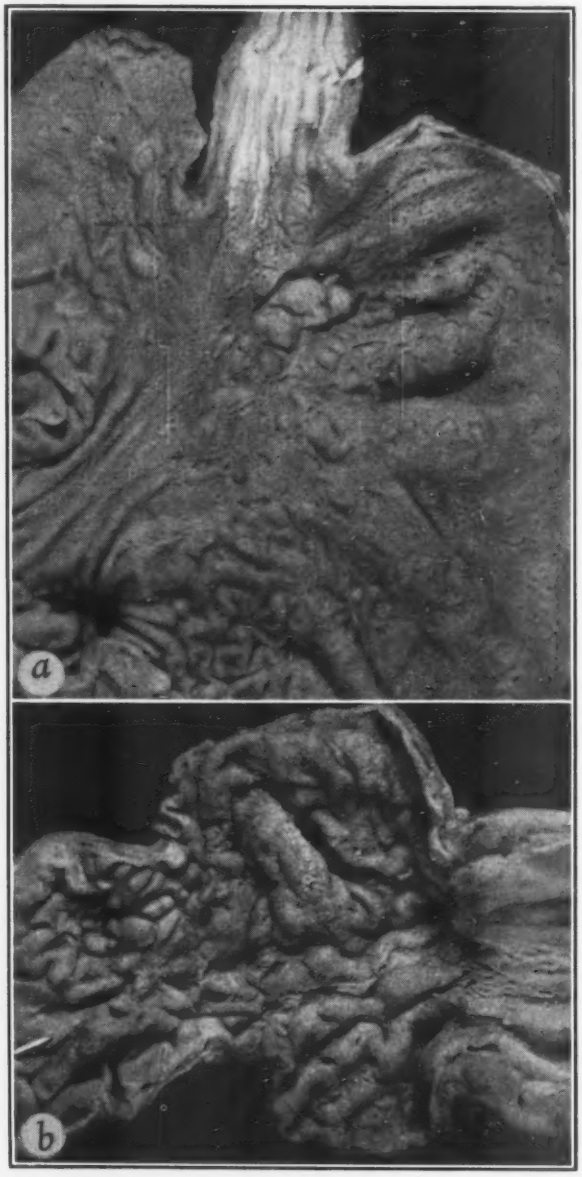


FIG. 3.—(Case 4). Ulcers and nodular thickening of the mucosa. *a*. Stomach. *b*. Duodenum.

at the junction of the 2nd and 3rd portions; the appearance of the constricting lesion was not neoplastic (Fig. 1*c*). The surgically formed stoma functioned in a satisfactory manner. The concentration of indirect-reacting bilirubin in the serum was 0.2 mg. per 100 cc. A sulfobromophthalein test of hepatic function revealed a retention of dye, grade 1 (6%), at the end of 1 hour. Gross and microscopic examinations of the feces revealed an excess of fat; occult blood was found in the stool, with both the guaiac and the benzidine tests.

Late in the evening on the 7th day in the hospital, the patient experienced a severe episode of upper abdominal pain which morphine sulfate relieved. Six hours later the pain returned and, while sitting on a bed pan, the patient suddenly collapsed and died within a very few minutes.

Necropsy. Slight emaciation was evident. The mesentery was studded with moderately firm lymph nodes measuring as much as 1.5 cm. in diameter. The spleen was enlarged and weighed 300 gm. The posterior gastrojejunal anastomosis was intact, and there was no ulcer at the stoma. The entire gastric mucosa, especially in the cardiac portion, was involved by multiple ulcerations (Fig. 3*a*). The largest ulcer measured 2.0 by 0.5 cm. and others were all 0.5 cm. in diameter or smaller. Between the ulcers there was a nodular hyperplasia of the mucosa with nodules measuring as much as 0.5 cm. in diameter. The pyloric muscle was hypertrophied and measured 0.5 cm. in thickness. The duodenum in its entirety was involved in the same type of process with multiple nodules of hyperplasia between the ulcers (Fig. 3*b*). The 3rd portion of the duodenum was markedly narrowed. The wall of the small bowel was slightly thickened. About 30 cm. proximal

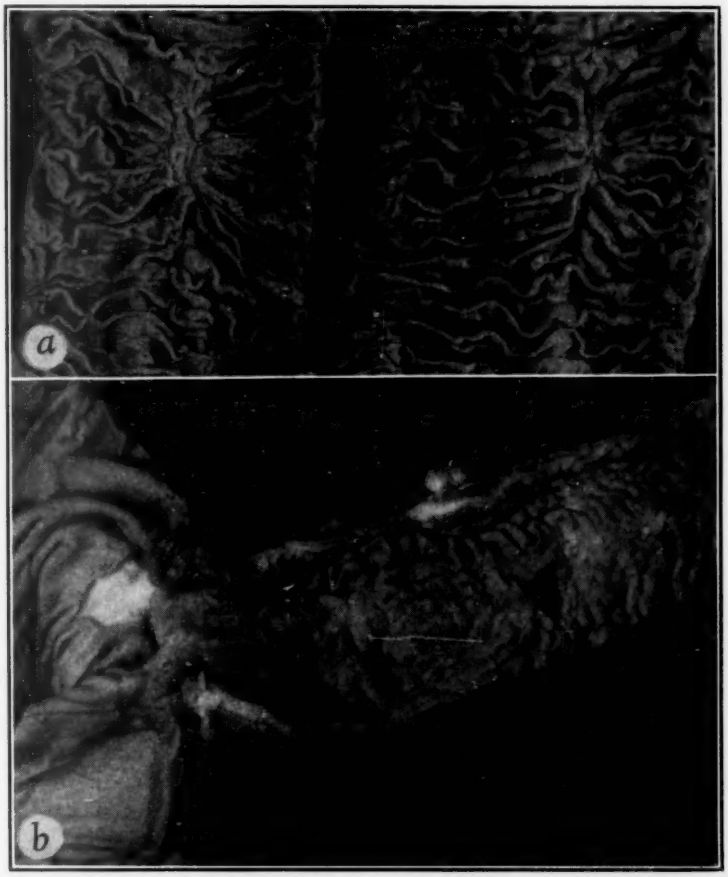


FIG. 4.—(Case 4). Regional enteritis. *a.* Jejunum. *b.* Terminal ileum and cecum.

to the ileocecal valve there was a group of ulcers, approximately 20 in number, which measured about 0.1 cm. in greatest diameter. Scattered throughout the ileum and in patchy areas of the jejunum proximal to this area there were numerous healing and active ulcers varying from 0.1 to 2.0 cm. in greatest diameter (Fig. 4*a*). In the region of the ileocecal valve and terminal ileum the wall was moderately thickened with nodular hyperplasia of the mucosa and numerous active and healing ulcers (Fig. 4*b*).

Both adrenal glands were found to be hemorrhagic and necrotic. On cross section, thrombi were found in both central veins.

Histologic Examination. In sections of the stomach some ulcers extended to the muscularis mucosa and in others this structure was destroyed. The base of the ulcers was covered by a fibrinopurulent exudate. Be-

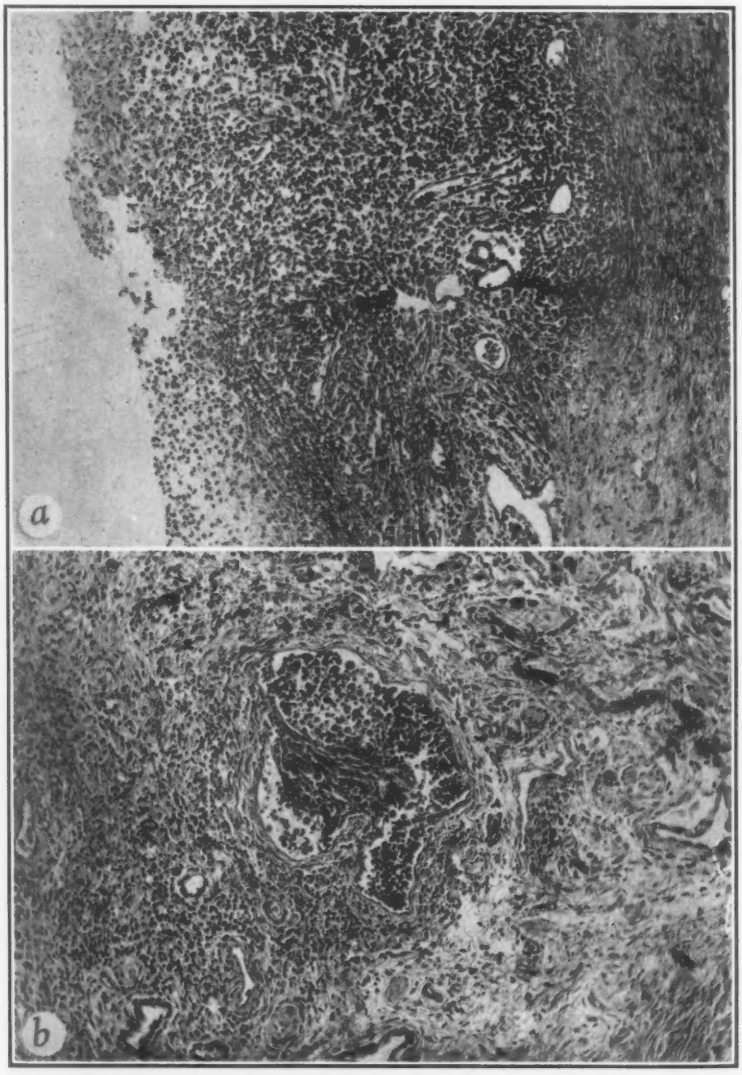


FIG. 5.—(Case 4). *a.* Ulcer of stomach with chronic granulomatous inflammation and dilated lymph spaces (hematoxylin and eosin, x 110). *b.* Submucosa of ileum showing leukocytes and fibrin in dilated lymph spaces (hematoxylin and eosin, x 100).

neath this exudate there was a granulomatous inflammation with giant cells. In the adjacent mucosa were many polymorphonuclear cells in the mucous glands, and in the interstitial tissue were increased numbers of plasma cells and lymphocytes. The epithelial cells of the mucous glands were hyperchromatic and actively proliferating. In the submucosa there were focal collections of lymphocytes. The lymphatics were markedly dilated and many contained lymphocytes (Fig. 5*a*). Sections through the ulcers found in the duodenum, jejunum and ileum were similar in appearance and will be described together. These ulcers extended down to the muscularis mucosae and in some instances down to the muscularis. The base of the ulcer usually was covered by a fibrinopurulent exudate. The adjacent mucosa and submucosa were infiltrated with lymphocytes, plasma cells and eosinophilic leukocytes. The lymph spaces of the submucosa was frequently filled with lymphocytes and fibrin (Fig. 5*b*). Scattered throughout the submucosa and the muscularis

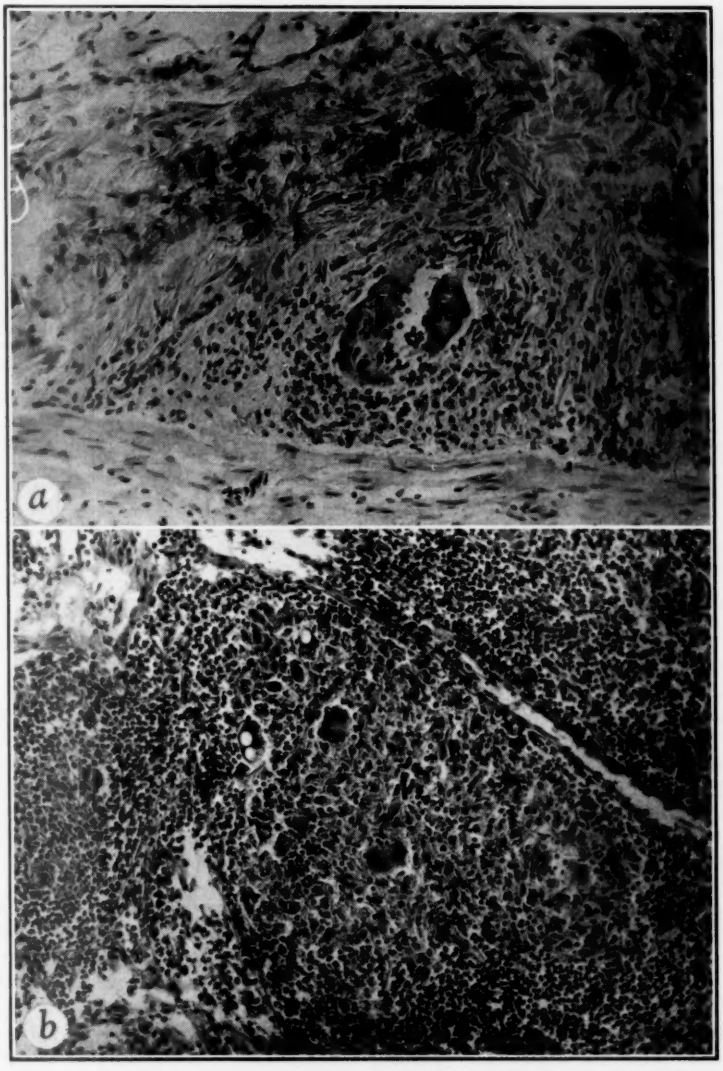


FIG. 6.—(Case 4). *a.* Submucosa of jejunum; granulomatous inflammation with giant cells (hematoxylin and eosin, x 135). *b.* Mesenteric lymph node (hematoxylin and eosin, x 125).

there were granulomas consisting of fibroblasts, lymphocytes and giant cells (Fig. 6*a*). Sections appropriately stained failed to reveal any acid-fast organisms.

In the liver the parenchyma showed numerous small granulomatous foci. These consisted of fibroblasts, lymphocytes and an occasional polymorphonuclear cell. The few liver cells which could be seen in these granulomas were atrophied or necrotic.

In the mesenteric and periaortic lymph nodes were numerous epithelioid-cell tubercles with giant cells of Langhans' type (Fig. 6*b*). Caseation or necrosis was not apparent in any of them. An occasional fibrotic scar was observed. Sections appropriately stained failed to reveal any acid-fast organisms.

Sections of both adrenal glands were similar in appearance and will be described together. They were completely necrotic and hemorrhagic except for a few foci of cortical cells adjacent to the capsule. The central vein contained an *ante mortem* thrombus completely blocking the lumen. In the periadrenal adipose and connective tissue there was a granulomatous type of inflammatory process. Fibroblastic proliferation, newly-formed capillaries, phagocytes, plasma cells, lymphocytes and an occasional giant cell were observed. A small periadrenal artery revealed an organized and canalized thrombus.

In the left lower lobe of the lung there were foci of organized pneumonia.

Anatomic diagnoses: chronic granulomatous and ulcerative gastro-enteritis; thrombosis of central veins of adrenal glands with recent infarction and adrenal insufficiency (clinical diagnosis); former posterior gastro-enterostomy for obstructing duodenal lesion; hypertrophy of spleen (weight 300 gm.; normal 150 gm.).

Case 5. A man, 36 years old, first came to the clinic in July, 1931. For the preceding 3 years he had experienced episodes of painless diarrhea at intervals of several months, with 4 to 6 bowel movements each 24 hours, for periods of 2 to 5 days. Physical examination revealed no significant abnormalities. Study of a fresh stool specimen revealed no pathogenic ova or parasites. He was dismissed without a specific diagnosis being made.

The patient next was seen 13 years later, in September, 1944, at which time he was 49 years of age. He was referred by his physician because of a tentative diagnosis of a gastric malignant lesion based on evidence of roentgenologic examination. The patient stated that he had had for the preceding 12 years, pressure in the middle of the epigastrium and right upper part of the abdomen and fullness after overeating, especially of fatty foods and that episodic diarrhea had persisted through the years. During the past 2 or 3 years the episodes of diarrhea, each lasting several days, were characterized by 8 to 10 watery movements for each 24 hours, both diurnal and nocturnal. During the preceding 4 months his distress was least evident before meals and was aggravated by eating. It had, however, disturbed his sleep. He was often nauseated but vomited only infrequently. During the preceding year, anorexia had been progressive and he had lost 35 lbs.

His weight was 110 lbs., unclothed. Blood pressure in mm. of mercury was 98 systolic and 60 diastolic. Wasting was obvious, and a peculiar, generalized, swarthy pigmentation of the skin was noted. A moderate degree of midepigastric tenderness was elicited by pressure but no abdominal masses or organs were palpable. The value for hemoglobin was 12.0 gm. per 100 cc. Erythrocytes numbered 3,580,000 and leukocytes 7,900. A stained blood smear revealed mild generalized macrocytosis but no hypersegmentation of the neutrophilic leukocytes. The sedimentation rate was slow. Roentgenographic examination of the thorax revealed minimal fibrosis at the left pulmonary apex; that of the stomach and duodenum revealed a constricting deformity of the 1st and 2nd portions of the duodenum (Fig. 1*d*). Morning aspiration of the fasting content of the stomach obtained 500 cc. Withdrawal of fractions during a histamine test revealed no free hydrochloric acid in a period of 90 minutes.

Exploratory laparotomy was carried out on the 12th day in the hospital. When the peritoneal cavity was opened, a small amount of free fluid was encountered. Inspection of the first and second portions of the duodenum and of the adjacent pylorus revealed much edematous swelling and inflammatory reaction. A longitudinal incision through the pyloric ring and the first portion of the duodenum showed a friable, easily bleeding, edematous duodenal wall. A specimen was removed from this area. The incision was closed in the long axis of the duodenum, then posterior gastroenterostomy was carried out. The gastric wall in the region of the stoma was considerably thickened.

Careful study of fixed and stained sections of tissue from the duodenal wall (Fig. 7) revealed ulceration down to the submucosa and in some areas down to the muscularis. The bases of these ulcers were covered by a fibrinopurulent exudate. Throughout the adjacent duodenal wall there were increased

numbers of lymphocytes, plasma cells and eosinophilic leukocytes. The submucosa in particular was markedly thickened by massive infiltration of these cells and numerous fibroblasts. The nerve plexuses of Auerbach and Meissner appeared to be hypertrophied. There were also numerous but scattered groups of epithelioid cells usually surrounded by a zone of lymphocytes. Giant cells of Langhans' type were encountered occasionally. Careful study of numerous sections stained for acid-fast bacilli failed to reveal any tubercle bacilli.

Postoperatively, the abdominal symptoms temporarily disappeared, but the exhaustion persisted. Concentrated liver extract was given intramuscularly and a low fat diet was employed because of the macrocytosis and the demonstration that 58% of the dry weight of a 24 hour collection of feces was fat. The concentration of serum protein was 4.9 gm. per 100 cc. with an albumin-globulin ratio of roughly 1:1. The concentrations of serum calcium and phosphorus were 8.0 and 2.9 mg. per 100 cc., respectively. A tuberculin test, with purified protein derivative 0.00002 mg. gave negative results. Roentgenologic examination of the stomach and small intestine revealed gastric dilatation (Fig. 8*a*). The constricting lesion of the 1st and 2nd portions of the duodenum appeared less marked in degree than it did preoperatively. Changes in the relief pattern of the mucous membrane at the gastrojejunal anastomosis were interpreted as jejunitis and a change in the motility of the small bowel similar to that seen in the so-called deficiency states was noted (Fig. 2*b*). Roentgenologic examination of the large intestine failed to reveal abnormality in the colon and terminal ileum. At dismissal in December, 1944, 2½ months following admission, he was advised to follow a high carbohydrate, high protein, low fat but high calorie dietary program, supplemented with intramuscular injections of liver extract and oral administration of vitamin concentrates. His weight at dismissal was 111 lbs.

The patient next was seen in June, 1945. He had gained 23 lbs., but continued to be bothered with upper abdominal fullness, pressure, belching and occasional nausea. The diarrhea had not recurred. The blood pressure was 96 systolic and 64 diastolic. The only significant findings were a moderate degree of generalized abdominal distention and tympany. The value for hemoglobin was 13.3 gm. per 100 cc., the erythrocyte count was 4,260,000 and the leukocyte count was 9,700. The sedimentation rate was 10 mm. in 1 hour (Westergren method). Roentgenographic examination of the thorax revealed minimal fibrosis in the left pulmonary apex, identical to that seen at his earlier examination. Roentgenologic examination of the upper part of the gastrointestinal tract revealed that the pylorus and the 1st and 2nd portions of the

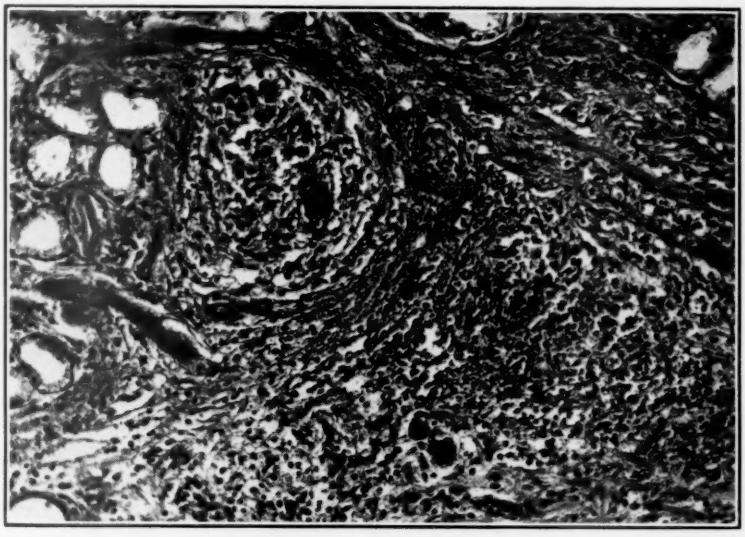


FIG. 7.—(Case 5). Specimen from duodenum showing granulomatous inflammation, tubercles and giant cells (hematoxylin and eosin, x 140).

duodenum were deformed as they were at the time of the original examination (Fig. 8*b*). The stoma of the gastro-enteric anastomosis was small, there was evidence of organic contraction of the jejunum at the anastomotic level, and transit of the contrast fluid was delayed. Roentgenologic examination of the colon and terminal ileum revealed no evidence of abnormality. The concentration of serum protein was 6.6 gm. per 100 cc. with an albumin-globulin ratio of 1.7:1. The patient was dismissed with instructions to continue use of the previously outlined program of therapy.

He was seen again in October, 1945, at which time he had gained a further 31 lbs., weighing 165 lbs. His complaints were essentially unchanged, though mild for the most part. He had had an occasional episode of diarrhea in the preceding 4 months. Physical findings, laboratory data and roentgenologic examinations of the gastrointestinal tract (Fig. 8*c*) were essentially as they were at the preceding visit. Tuberculin test again gave negative results with the first strength purified protein derivative, but there was a faintly positive skin test when the second

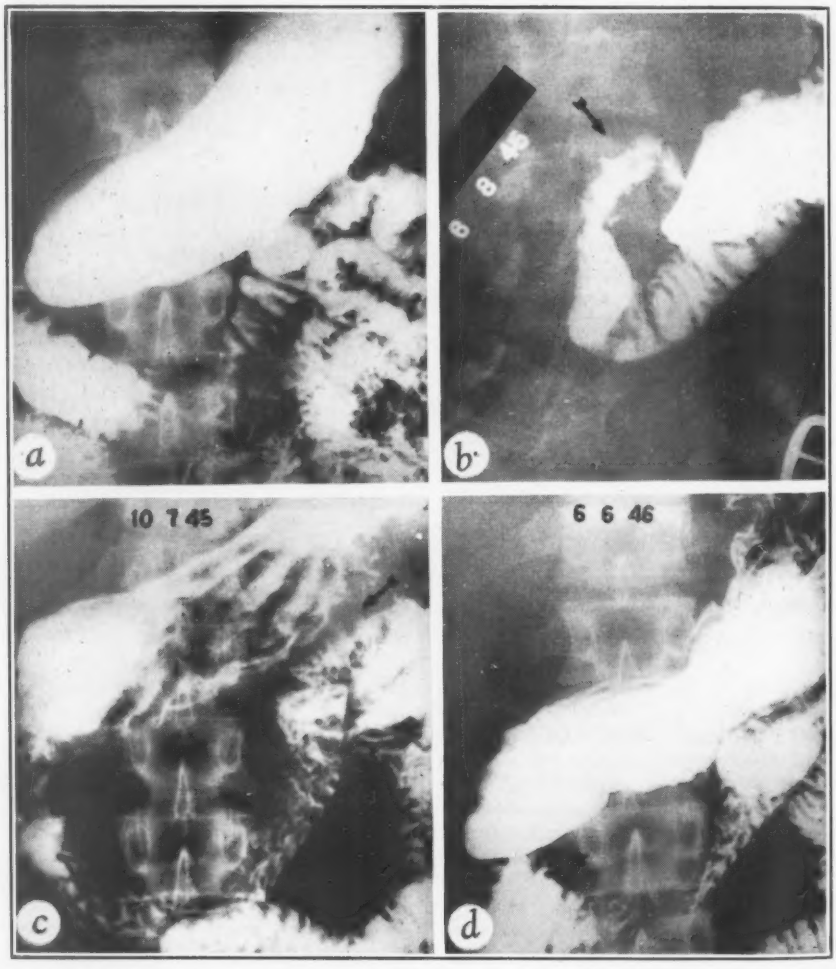


FIG. 8.—(Case 5). The duodenum in: *a*, September, 1944, *b*, June, 1945, *c*, October, 1945, *d*, June, 1946, showing the persistence of the deformity in the first and second portions. The arrows pointing to the gastrojejunal anastomosis indicate the site of the short constricting deformity of the jejunum in that region; this was considered to be evidence of ulcerative jejunitis; this deformity also persisted almost unchanged from September, 1944 to June, 1946.

strength purified protein derivative, 0.005 mg. was given intracutaneously.

On re-examination in May, 1946, the patient's condition was unchanged. Essentially identical roentgenologic deformities in the duodenum and in the jejunum at the anastomotic level to those previously described were observed (Fig. 8*d*).

A letter in March, 1947, revealed no particular change in his reported state of health. There had been an occasional episode of diarrhea but no change in weight.

Comment. These 5 cases of inflammatory disease of the stomach, duodenum and jejunum have much in common clinically, roentgenologically and pathologically. Clinically, 4 features are outstanding: 1, continuous and intermittent upper abdominal distress intensified by the ingestion of food, loss of weight and strength, nausea and occasional vomiting occurred in several cases. 2, diarrhea, steatorrheal and episodic in type, occurred in Cases 2 to 5, inclusive. The diarrhea was not always associated with pain. 3, gastric retention, denoted by a succussion splash and by morning aspiration in the fasting state of larger than normal amounts of gastric contents, occurred in Cases 2 to 5, inclusive. 4, evidence of deficient absorption also was observed in Cases 2 to 5, inclusive. This evidence included macrocytic anemia (Cases 3 and 5), hypolipemia (Case 2), hypoproteinemia (Cases 3 and 5), hypocalcemia (Case 5), hypoprothrombinemia (Case 3), and a flat curve on glucose tolerance test (Case 3). Clinically, the association of diarrhea with symptoms of upper gastrointestinal obstruction and with evidences of deficient absorption suggested a lesion producing obstruction or stasis of considerable extent, sufficient to produce measurable interference with absorption from the small bowel.

Roentgenologically, the picture was that of disturbed motility of one or more portions of the upper part of the gastrointestinal tract, with or without persistent or transitory constriction and dilatation. Thus, atony and ineffective peristalsis were noted in Cases 2 and 4, slowed emptying and dilatation of the stomach in Cases 2 and 4 with retained secretion in Cases 2 and 4. Constriction of the 1st, 2nd or 3rd portions of the duodenum were found in Cases 2, 3, 4 and 5. Constrictions were tubular without sharply demarcated orad and caudad extremities. Within the constrictions the relief pattern of the mucous membrane was markedly subdued or absent. The constrictions were persistent in Cases 4 and 5, inconstant in Case 3. In Case 1 there was roentgenologic evidence of change in the mucosa, and in Case 4 ineffective peristaltic activity of the duodenum. In Case 1 the motility of a slightly dilated jejunum was abnormal, and in Case 5 organic contraction of the jejunum at anastomotic level was noted.

Roentgenologically the inflammatory process involved the jejunum in Case 1, the duodenum in Cases 3 and 5, and the stomach and duodenum in Cases 2 and 4, but actually it was not or did not remain limited to these areas. In Case 1 an abnormal mucosal pattern was noted in the duodenum 6 months after the initial examination.

In Case 2 the surgeon noted involvement of the jejunum but not of the stomach and duodenum, and in Case 3 of the jejunum as well as the duodenum. In Case 5, at a late date, there was roentgenologic evidence of involvement of the jejunum as well as of the duodenum (the surgeon had noted involvement of the stomach as well as the duodenum but not of the jejunum in this case) and in Case 4, necropsy 3 years after the roentgenologist and surgeon had found involvement of only the stomach and duodenum, disclosed widespread involvement not only of the stomach and duodenum but also of the entire small

bowel, mesenteric nodes and periadrenal tissues. In Cases 2, 4 and 5 the inflammatory process appears to have ultimately involved areas of the small bowel commonly involved in regional enteritis.

Gastroscopy was carried out only in Case 2. Gastroscopy revealed diffuse superficial gastritis with multiple areas of superficial ulceration.

Surgical exploration in each case disclosed an inflammatory process, involving the upper part of the gastrointestinal tract and varying in extent and severity. The findings are similar. In Case 1, the walls of the proximal 8 to 10 feet of the jejunum were thickened and edematous; the lumen of the involved jejunum was enlarged 3 to 4 times normal size, the mesentery of the small bowel was edematous; free fluid was present in the abdominal cavity. In Case 5 the gastric walls were thickened, the pylorus and the walls of the 1st and 2nd portions of the duodenum were inflamed and edematous; free fluid was noted in the abdominal cavity. The constriction of the 1st and 2nd portions of the duodenum, noted at roentgenologic examination, conformed to the area of edematous thickening of the duodenum. In Case 2, large inflamed omental nodes were found, and on opening the jejunum its mucosa was noted to be granular and edematous but the roentgenologic findings of dilatation of the stomach, duodenum and jejunum and of constriction at the duodenojejunal junction was not confirmed. In Case 4 the pylorus, the walls of the stomach and of the first part of the duodenum were thickened and edematous; a second area 2 to 3 cm. distal to the papilla of Vater was noted; the mesenteric nodes were large; the jejunum and ileum appeared normal.

In Case 3 the duodenum and upper part of the jejunum were the seat of a mild, diffuse inflammation. Mesenteric nodes were enlarged; the inconsistent constriction of the 3rd portion of the duodenum, noted by the roentgenologist, was not found. The terminal ileum was normal. At operation, then, the inflammatory process involved the stomach in Cases 4 and 5, the duodenum in Cases 3, 4 and 5, the jejunum in Cases 1, 2 and 3, the mesentery in Case 1 and the mesenteric or omental nodes in Cases 2, 3 and 4.

Histologic study of material from the wall of the pylorus and duodenum in Case 5, from the wall of the jejunum in Case 2 and from the wall of the stomach in Case 4, together with a mesenteric node in Case 5 disclosed an inflammatory process. Further study of the material removed in Case 5 and study of the material removed at necropsy in Case 4 disclosed that the inflammatory process was of the nonspecific granulomatous type seen in regional enteritis.

The tubercles in particular were identical with those described and regarded as pathognomonic of regional enteritis by Hadfield.[6] The inability to find bacilli of tuberculosis in these lesions lends further support for the diagnosis of regional enteritis. It is of interest to note in this connection, although unrecognized clinically, that segmental ulcerative jejunitis and terminal ileitis were found at necropsy in Case 4. In this case also we apparently have the first reported pathologically verified instance of a granulomatous inflammatory process in the stomach identical with that observed in regional enteritis. The ulceration, the granulomatous type of inflammation, the inflammation and obstruction of the lymph vessels, were like those observed in the terminal ileum in the same case. Cases 4 and 5 likewise substantiate Hadfield's contention that in histopathologic appearance the lesions more closely resemble

Boeck's sarcoidosis than tuberculous infection. As he pointed out, there is the same tendency to spontaneous retrogression and inconspicuous healing, and in neither disease has a specific infectious agent been isolated. Case 4, furthermore, gives some support to Hadfield's contention that if cases of regional enteritis are followed for a sufficiently long period, evidence of generalization, as in Boeck's sarcoidosis, will be found. In Case 4, generalization had occurred to the mesenteric and periaortic lymph nodes, to the periadrenal connective tissue and possibly also to the liver. In the latter organ the granulomatous foci did not have the typical appearance of those in the intestinal tract or lymph nodes, but this may have been due to the fact that the lesions were in an earlier stage of development. The pathologic findings in cases of regional ileitis do not always resemble those of sarcoidosis. Thus Watson, Rigler, Wangensteen and McCartney[13] in studying pathologic material in 23 cases of regional ileitis found that in only 2 cases was the pathologic process typical of sarcoidosis. Dockerty[5] has estimated that 25% of the specimens removed at operation for regional enteritis reveal the histologic appearance of sarcoidosis.

Recognition that nonspecific granulomatous inflammation characteristic of regional enteritis involves the stomach invites careful re-examination of the pathogenic nature of ulcerative gastritis, benign linitis plastica and tuberculosis of the stomach in certain cases.

Failure to recognize clinically Cases 2 to 5 inclusive as examples of regional enteritis is not unexpected because, as has been noted, a region of the gastrointestinal tract not commonly known to be affected by the disease was involved. However, in each case the disease involved, at the time of the initial examination or subsequently, areas of the gastrointestinal tract commonly involved in regional enteritis. In Cases 4 and 5 the pathologic process eventually was identified as regional ileitis. Similarity of the clinical, roentgenographic and surgical findings in Cases 1, 2, 3, 4 and 5 justifies the assumption that the pathologic processes in all these cases are similar and like that of regional enteritis.

In our experience, nonspecific granulomatous gastroenteritis in which the process is limited clinically to the upper part of the gastrointestinal tract must be distinguished from idiopathic steatorrhea, as it must be when the jejunum and ileum are involved. The diagnosis of idiopathic steatorrhea is suggested to the clinician by the combination of steatorrheal diarrhea with one or more of the several evidences of a deficiency of absorption in these cases. However, the recurrent attacks of abdominal pain with evidences of stasis or obstruction of the upper part of the intestine, as well as roentgenologic evidence of motor dysfunction, gastric retention, localized areas of persistent dilatation and constriction served to differentiate the condition from idiopathic steatorrhea. These cases serve to emphasize the necessity of complete gastrointestinal roentgenologic study in all cases of steatorrhea with multiple deficiencies to avoid confusion between idiopathic steatorrhea and nonspecific granulomatous gastroenteritis.

Differentiation of nonspecific granulomatous inflammation of the upper part of the gastrointestinal tract from duodenal ulcer, or from neoplasm of the duodenum should present no difficulty. Neoplasms of the duodenum produce obstruction but in our experience not the diarrhea, the deficiency state, or the roentgenologic evidence of dysfunction.

Summary and Conclusions. The non-

specific granulomatous inflammation of the intestinal tract commonly designated as regional enteritis may involve the stomach and duodenum as well as the jejunum and ileum, and more appropriately, may be designated as nonspecific granulomatous gastroenteritis. The stomach and duodenum may be involved when the small bowel is the major seat of involvement or may be more markedly involved than the small bowel.

In the cases of nonspecific granulomatous gastroenteritis studied thus far, in which the clinical and roentgenographic involvement apparently is limited to the stomach and duodenum or duodenum, the process has provoked recurring episodes of upper abdominal pain and diarrhea with clinical and laboratory evidences of stasis or obstruction, and of disturbance of intestinal absorption, steatorrhea, macrocytic anemia, hypolipemia, hypoprothrombinemia, hypocalcemia and flattening of the glucose tolerance curve. Roentgenologically, the findings of impaired motility with transitory or especially permanent constriction serve to distinguish this condition from idiopathic steatorrhea.

REFERENCES

1. **Bargen, J. A.**: Proc. Staff Meet., Mayo Clin., **13**, 550, 1938.
2. **Brown, P. W., Bargen, J. A.**, and **Weber, H. M.**: Am. J. Dig. Dis., **1**, 426, 1934.
3. Case Record 35171: Mass. Gen. Hosp.: New England J. Med., **240**, 692, 1949.
4. **Crohn, B. B., Ginzburg, L.**, and **Oppenheimer, G. D.**: J. Am. Med. Assn., **99**, 1323, 1932.
5. **Dockerty, M. B.**: Personal communication.
6. **Hadfield, G.**: Lancet, **2**, 773, 1939.
7. **Harris, F. I., Bell, G. H.**, and **Brunn, H.**: Surg., Gyn. and Obst., **57**, 637, 1933.
8. **Mock, H. E.**: Ibid., **52**, 672, 1931.
9. **Moschcowitz, E.**, and **Wilensky, A. O.**: Am. J. Med. Sci., **166**, 48, 1923.
10. **Ross, J. R.**: Gastroenterology, **13**, 344, 1949.
11. **Shapiro, R.**: Am. J. Med. Sci., **198**, 269, 1939.
12. **Warren, S.**, and **Sommers, S. C.**: Am. J. Path., **24**, 475, 1948.
13. **Watson, C. J., Rigler, L. G., Wangensteen, O. H.**, and **McCartney, J. S.**: Gastroenterology, **4**, 30, 1945.

MENINGITIS DUE TO PSEUDOMONAS AERUGINOSA TREATED WITH POLYMYXIN B

By E. R. Hayes, M.D.

AND

Ellard Yow, M.D.*

MINNEAPOLIS, MINNESOTA

(From the Department of Internal Medicine, University of Minnesota Hospital)

Infections due to *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*) remain an important therapeutic problem, since available chemotherapeutic agents are frequently ineffective. This problem is particularly important in infections of the meninges because of the high mortality rate in untreated cases. In a review of the reported cases of *Pseudomonas* meningitis up to 1947, Stanley[10] found 41 cases of primary infection of the meninges and 29 cases in which the meningitis was secondary to a focus elsewhere. Of the cases of primary meningitis, 32 were due directly to the performance of a lumbar puncture, either for diagnostic purposes or for the injection of solutions into the subarachnoid space. Treatment in these cases consisted of repeated spinal drainage and sulfonamide administration. The mortality rate in the cases of primary infection was 55%, and 86% of those patients in which the infection resulted from *Pseudomonas* bacteremia died.

In a review of the first 3000 cases treated with streptomycin, the Committee on Chemotherapy of the National Research Council[6] reported 11 cases of meningitis due to *Pseudomonas aeruginosa*. In 7 of these cases the infection was definitely related to lumbar puncture. Two of the patients were being given penicillin intrathecally; in 4 instances meningitis followed a lumbar puncture done for diagnostic purposes, and in 1 case lumbar puncture was performed for the administration of spinal anesthesia. The infection in 2 of these patients was from erosion of a cervical meningocele and an open spina bifida. In the 2 remaining patients the meningitis was secondary to a *Pseudomonas* bacteremia.

All 11 patients, except 1, had received sulfonamide therapy without definite beneficial effect. Streptomycin was given intramuscularly, and in all but 1 case, intrathecally. The mortality rate in this group of patients was 36%.

No cases of *Pseudomonas* meningitis treated with aureomycin or chloromycetin have been reported. Urinary infections due to *Pseudomonas aeruginosa* have been treated with these antibiotic agents, but the results of therapy have generally been unfavorable[9].

A recently isolated polypeptide antibiotic, polymyxin, has been found to be active exclusively against gram negative bacteria. It is composed of 5 fractions (A, B, C, D, and E), all having very similar chemical and biological properties[4]. Polymyxin A was originally isolated from *Bacillus aerosporus* by Ainsworth, Brown, and Brownlee[1] and was called aerosporin. Polymyxin D was described by Benedict and Langlykke[2], and by Stansly, Shepherd, and White[11]. It

* Now at Baylor University College of Medicine, Houston, Texas.

was isolated from *Bacillus polymyxa*. As a result of studies designed to purify further these antibiotics and to decrease their toxicity, the other fractions were isolated, differing from each other in amino acid content[5].

Experimental studies by Bliss, Chandler, and Schoenbach[3] revealed that polymyxin is a bactericidal drug active against *Aerobacter aerogenes, E. coli, Sal. typhosa, K. pneumoniae, H. influenzae,* and *Pseudomonas aeruginosa,* but not against *Proteus vulgaris.*

Clinical studies with polymyxin have been reported by Schoenbach, Bryer, and Long[8]. Twenty-two patients were treated: 5 with pertussis, 4 with brucellosis, 4 with typhoid fever, 1 with peritonitis, 3 with infections due to *Pseudomonas aeruginosa,* 3 with infections due to *Aerobacter aerogenes,* and 2 with infections due to *K. pneumoniae.* Results were described as excellent in those infections due to *Pseudomonas aeruginosa,* good in the others except for the patients with typhoid fever, in which cases the results were variable. Pulaski, Baker, Rosenberg, and Connell[7] reported the use of polymyxin B and E in 20 patients with urinary infections and in several patients with granulating wounds infected with *Pseudomonas aeruginosa.* The results in these patients were very good.

The results of these experimental and clinical studies prompted the trial of polymyxin in a case of chronic meningitis due to *Pseudomonas aeruginosa* failing to respond to sulfonamides, penicillin, streptomycin and aureomycin. The treatment was successful.* The patient was desperately ill and the course of her disease was unaffected by sulfonamide and antibiotic therapy, until polymyxin was administered. Following therapy, she has remained well for 7 months.

Case Report. M. H., a 17 year old white girl, was admitted to the University Hospital on February 3, 1949, and discharged on June 28, 1949 (See Fig. 1).

Recent Illness. During the fall of 1948 she had had 3 or 4 attacks of abdominal pain, sharp in character and generalized over the abdomen. These attacks seemed to come on in the evening, to last for several hours, and would usually be relieved by vomiting. The last attack was in November, 1948. During this attack, she was hospitalized for a short period of observation and it was concluded that she had acute appendicitis; consequently, an interval appendectomy was performed on December 17, 1948, using spinal anesthesia. The postoperative course was uneventful until 3 days following the operation, when she developed a severe generalized pounding headache and a fever of about 99.5°F. The headache lasted 2 to 3 hours. She had no recurrence of headache, but continued to have low-grade fever for the next 4 days. She was discharged from the hospital on December 24 with a low-grade fever. She had no particular complaints immediately following discharge, but on the 5th day after release her temperature rose to 102°F. and she developed backache, headache, and pain between the shoulders. She was readmitted to the hospital at this time and a spinal tap was done, which revealed "a few cells," but no organisms were identified; no culture was taken. Following the spinal puncture, she was placed upon penicillin, streptomycin, sulfadiazine, and aureomycin. Six intrathecal injections of penicillin were given at this time; however, she continued to have fever up to 103° every night. She remained in the hospital until January 29, at which time the symptoms had cleared, but some fever persisted. On January 31, she again developed severe headache, backache, and pain in both shoulders. Shortly thereafter, she vomited and the symptoms were relieved; however, she continued to be febrile.

The *past history* was not remarkable, and her general health had been good prior to her present illness.

Physical examination, at the time of admission to the hospital, revealed a well developed, well nourished white girl who did not appear acutely or chronically ill. Her temperature was 99.8, her pulse 108, respiration 20,

*The polymyxin B used was kindly supplied by Dr. Emanuel B. Schoenbach, of the Department of Preventive Medicine, Johns Hopkins University School of Medicine and was manufactured by Burroughs Wellcome & Co., New York, N. Y.

and blood pressure 120/75. She was found to have a very slight limitation of anterior flexion of the neck and absent abdominal reflexes. Otherwise the physical examination was entirely within normal limits.

Laboratory examinations, on admission, were as follows: The urine had a specific gravity of 1.020 and was acid in reaction. The sediment contained only occasional sulphadiazine crystals. The hemoglobin was 12.7 gm. The leukocyte count was 12,600 (69% neutrophils, 22% lymphocytes, 5% monocytes, and 4% eosinophils). The erythrocyte sedimentation velocity was 33 mm. (Westergren). Repeated cultures of blood remained sterile. Urine cultures, on 2 occasions, revealed *E. coli* and non-hemolytic streptococci. Agglutination tests for S. *typhosus* were reported atypically positive in a dilution of 1:80; for brucella, atypically positive 1:40; and for paratyphoid organisms, atypically positive 1:80. A Mantoux test in a dilution of 1:1000 was negative.

The night following admission to the hospital, the patient's temperature rose to 102°. She was afebrile for the next 2 days. Then on the 4th hospital day, February 6, she had a chill and a rise in temperature to 103.6°. On February 7, there was a questionable increase in rigidity of the neck with a positive Kernig's sign. She continued to have daily fever. On February 8, neurological examination was essentially normal, except for a suggestive inequality of the knee jerks and adductor reflexes.

On February 10, the patient was noted to have a slight blurring of the right optic disk margin and a lumbar puncture was done. This was 26 days following the last spinal puncture. The fluid was under a pressure of 25 mm. of mercury. There were 760 white cells per cmm. (47% neutrophils). Following this lumbar puncture, the patient was given penicillin, 100,000 units intramuscularly every 3 hours and 500,000 units intravenously every 12 hours. During the next 2 days there was a rapid progression of abnormal neurological findings. The patient developed a 2 diopter choke on the right side with a ½ to 1 diopter on the left. The ankle jerk became

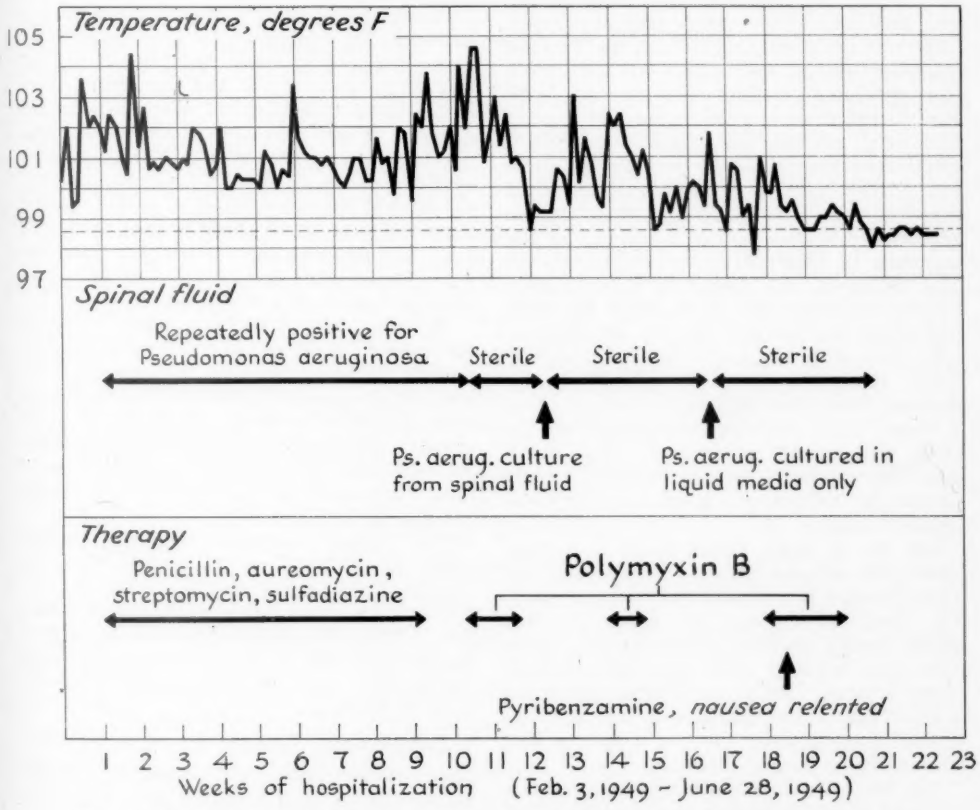


FIG. 1.—Clinical Course in a case of meningitis due to *Pseudomonas aeruginosa.*

exaggerated on the right and the knee jerk exaggerated on the left. The toe signs were questionably positive on the left. On February 14, the choked disk was receding and the patient was complaining less of headache, although she had definitely positive toe signs on the left side.

In spite of this apparent clinical improvement, the patient continued a febrile course with occasional chills. On February 15, aureomycin was administered in doses of 200 mg. intramuscularly 4 times daily. This was continued until February 17, at which time she was able to retain the aureomycin by mouth, and she received 250 mg. every 4 hours. During this period, sulfadiazine and penicillin were being administered. Dihydrostreptomycin, 0.5 gm. every 8 hours, was started on March 3. Intrathecal dihydrostreptomycin, in 50 mg. doses, was started March 17 and was given daily through March 23, at which time the dose was increased to 100 mg. daily until April 3. Penicillin had been discontinued on March 9, 1949.

Pseudomonas aeruginosa was repeatedly cultured from the cerebrospinal fluid. One of the cultures was tested for sensitivity to various antibiotics. The results were as follows: there was beginning inhibition with 25 μgm. of dihydrostreptomycin per ml. and complete inhibition at 100 μgm. per ml. With aureomycin, there was beginning inhibition at 12.5 μgm. per ml. and complete inhibition at 25 μgm. per ml. There was no inhibition with 10,000 units of penicillin per ml. Over a period of 2 weeks of therapy, there was no appreciable change in the sensitivity of the organism to these various antibiotic agents. The organism was found to be extremely sensitive to polymyxin B. On April 15, 1949, polymyxin was started, 80 mg. intramuscularly every 6 hours and 2 mg. intrathecally every 12 hours. On April 16, the intramuscular dose was reduced to 40 mg. every 4 hours. This was continued until April 25, 1949, with a total intramuscular dose of 2.2 gm. The cerebrospinal fluid became sterile on the day following institution of the polymyxin therapy.

On May 2, 1949, *Pseudomonas aeruginosa* was again cultured from the spinal fluid. The patient began to exhibit fever again at about this time and therapy with polymyxin was again started on May 13, 1949. The drug was given in a dose of 40 mg. intramuscularly every 4 hours, with 2 mg. intrathecally twice a day. This was discontinued on May 17, after a total intramuscular dose of 1.12 gm. and an intrathecal dose of 28 mg.

On May 28, *Pseudomonas aeruginosa* was cultured in liquid media only from the spinal fluid. Another culture on May 31 remained sterile. The patient continued to have elevation of temperature to 101°. A 3rd course of polymyxin was started on June 6, 1949, the patient receiving 40 mg. intramuscularly every 6 hours. She received 2 mg. intrathecally daily starting on June 6 and continuing through June 10, at which time intrathecal treatment was discontinued because of evidences of central nervous system irritation, with pains down the leg and in the back. Intramuscular administration was continued until June 22, the patient receiving a total of 2.64 gm. intramuscularly and 10 mg. intrathecally.

The use of polymyxin had been attended by considerable nausea and vomiting through all of its use, necessitating the administration of parenteral fluids. During the 3rd course of therapy, the patient developed generalized pruritus, for which pyribenzamine in 50 mg. doses was used. Coincident with the use of this medication, the pruritus stopped; thereafter the drug was administered before injection of polymyxin. On June 14, just after pyribenzamine was started, the patient had remission of the nausea and vomiting, so that she began to take nourishment by mouth.

Cerebrospinal fluid, obtained by lumbar tap on June 23, contained 12 leukocytes (1 a neutrophil and 11 mononuclears). This fluid was sterile on culture. Another specimen of fluid obtained on June 27 had 11 cells per c.mm., 4 of which were neutrophils. The protein content was 81 mg. per 100 cc. Cultures remained sterile. In the interval from June 13, the patient had eaten well and had gained from 103 pounds to 108½ pounds. She had been afebrile since June 10. At this point she was discharged from the hospital and remained under observation in the city.

She was seen on July 13, 1949, 7 months after onset, at which time she weighed 118 pounds. There had been no elevation in temperature since the time of her discharge from the hospital. She complained of some tinnitus and very mild hearing loss, which an audiogram showed to be of the nerve type. There were no other neurological abnormalities at that time.

The patient was again seen in October, 1949, at which time there had been no change in the audiogram. She had no complaints other than the hearing loss. By January, 1950, she had regained her original weight and felt well in all respects; even feeling that there had been some improvement in hearing.

Discussion. Several features of this case seem worthy of comment. Schoenbach, Bryer, and Long[8] noted that the most frequent complication encount-

ered in the use of polymyxin was renal toxicity. This patient developed albuminuria during the time she received polymyxin. Pulaski *et al.*[7] noted no toxicity following oral or topical application of the drug, but did note neurological disturbances such as paresthesias, hypesthesias, and mild dizziness, almost uniformly after the intramuscular administration of polymyxin. Our patient likewise developed these symptoms. Schoenbach, Bryer, and Long[8] noted nausea and vomiting in 4 of their patients. This symptom was prominent in our patient, but disappeared coincident with the administration of pyribenzamine. It would appear that this type of drug should be tried in further cases demonstrating toxicity to polymyxin.

The hearing loss is of interest. It was first noted 5 weeks after the last use of dihydrostreptomycin and between the 2nd and 3rd courses of polymyxin. The symptoms were not aggravated by the subsequent course of polymyxin and have remained stationary for 9 months since that time. One could raise the question as to whether the 8th nerve damage was a delayed effect of dihydrostreptomycin which involved the cochlear division of the nerve. However, in view of the nature of the illness which might have produced this sort of nerve damage, it would seem wise only to raise the question for future observation in patients treated with dihydrostreptomycin.

The incidence of meningitis due to a *Pseudomonas aeruginosa* infection is not common, but may occur secondary to spinal puncture or spinal anesthetic. The mortality rate is high in untreated cases, and the use of a drug known to be toxic seems justified until such time as a less toxic effective drug is available.

Conclusions. Evidence available at the present time indicates that polymyxin is superior to any other available drug in the treatment of infections due to *Pseudomonas aeruginosa*. Because of the toxicity of polymyxin, however, sulfadiazine and dihydrostreptomycin should first be administered simultaneously in adequate doses, and polymyxin given if these fail to control the infection.

REFERENCES

1. **Ainsworth, G. C., Brown, A. M.,** and **Brownlee, G.**: Nature, **160**, 263, 1947.
2. **Benedict, R. G.,** and **Langlykke, A. F.**: J. Bact., **54**, 24, 1947.
3. **Bliss, E. A., Chandler, C. A.,** and **Schoenbach, E. B.**: Ann. N. Y. Acad. Sci., **51**, 944, 1949.
4. **Brownlee, G., Bushby, S. R. M.,** and **Short, E. I.**: Ibid., **51**, 952, 1949.
5. **Jones, T. S. G.**: Ibid., **51**, 909, 1949.
6. **Keefer, C. S.,** and **Hewitt, W. L.**: The Therapeutic Value of Streptomycin: a Study of 3000 Cases, Ann Arbor, Mich., J. W. Edwards, 1948.
7. **Pulaski, E. J., Baker, H. J., Rosenberg, M. L.,** and **Connell, J. F., Jr.**: J. Clin. Invest., 28, 1028, 1949.
8. **Schoenbach, E. B., Bryer, M. S.,** and **Long, P. H.**: Ann. N. Y. Acad. Sci., **51**, 987, 1949.
9. **Spink, W. W.,** and **Yow, E. M.**: J. Am. Med. Assn., **141**, 964, 1949.
10. **Stanley, M. M.**: Am. J. Med., **2**, 347, 1947.
11. **Stansly, P. G., Shepherd, R. G.,** and **White, H. J.**: Bull. Johns Hop. Hosp., **81**, 43, 1947.

CULTURAL PROPERTIES AND PATHOGENICITY OF CERTAIN MICROORGANISMS OBTAINED FROM VARIOUS PROLIFERATIVE AND NEOPLASTIC DISEASES*

By Virginia Wuerthele-Caspé, M.D.

Eleanor Alexander-Jackson, Ph.D.

John A. Anderson, Ph.D.

James Hillier, Ph.D.

Roy M. Allen, D.Sc.

and

Lawrence W. Smith, M.D.

NEWARK, NEW JERSEY

(From the Bureau of Biological Research (Presbyterian Hospital Branch), Rutgers University)

Demonstration of the presence of specific microorganisms consistently occurring in tumor cells was first reported by Wuerthele-Caspé in 1947. These organisms exhibited cultural and morphologic features suggesting their possible relationship to the mycobacteria. A symposium reviewing these findings in some detail was published in 1948[9]. Prior to the observation and isolation of these acid-fast organisms from neoplastic tissues of animals and from man, the presence of similar microorganisms in generalized systemic sclerosis (scleroderma), a proliferative collagen disease, had been observed and reported[10]. Following these initial observations, further isolations were made from both blood and tissues of other sclerodermal and cancerous patients. The nature and pathogenicity of these organisms were reported in a preliminary study[7]. The widespread occurrence of these microorganisms in several types of proliferative diseases, and their successful culture outside the body in artificial media, emphasized the importance of a more detailed study of their characteristics. The present paper is an extension and continuation of the previous work.

Scleroderma Strain. The original cultures isolated on Petragnani and Lowenstein egg media from blood and lesions of patients with scleroderma revealed acid-fast granules of various sizes as well as rod-like forms. Electron-microscope studies suggest that the latter may be linear aggregates of the granular forms. Subcultures of the scleroderma organism taken from the original egg medium grew poorly on Sabouraud's agar. These organisms subsequently were isolated more satisfactorily in an especially devised liquid medium. These scleroderma strains have produced a consistent type of lesion when inoculated into white mice.†

* Aided by grants from the American Cancer Society, the Damon Runyon Fund, and the Abbott Laboratories of Chicago, Ill. Acknowledgement is made also for the support received from the Presbyterian Hospital, Newark, N. J.

† A report dealing more fully with the cultural properties and pathogenicity of the scleroderma organism as well as Alexander-Jackson's liquid medium will be presented at a later date.

HUMAN MALIGNANT STRAIN. Employing the technique developed in the scleroderma study by Alexander-Jackson and Wuerthele-Caspé[10], the latter isolated similar microorganisms from the blood of 18 cancer patients, 7 of whom had Hodgkin's disease, by inoculating their blood into chick embryos. Since the chicken has many similar appearing organisms occurring spontaneously, direct isolations were subsequently made on solid egg media. Unfortunately growth on this artificial medium proved to be feeble and scanty. It was not until Dubos', Alexander-Jackson's, and brain-heart infusion liquid media were used that the organisms could be isolated from human pathologic blood and tissues, and that sufficient growth could be obtained for cultural and pathogenicity studies. To date, no growth has been obtained from a similar control series of 17 normal individuals.

SPONTANEOUS ANIMAL TUMOR STRAINS. Concomitant with the study of isolates obtained from human cancer, isolations were also made repeatedly from Rous chicken sarcoma and Sarcoma 180 of the mouse. These cultures likewise reveal a consistent morphology similar to that of the microorganisms found in tissues from cases of human cancer. Although all of these organisms were morphologically similar, yet certain differences, such as rate of growth and size of granules, were found that may aid in their differentiation from one another. The Rous chicken sarcoma cultures were obtained on many media, either from the blood of chickens inoculated with tumor tissue or from the tumor directly. The Rous cultures grew on egg media, blood agar, Dubos' medium, Petroff's medium and other enriched agars. The growth appeared in from 3 to 7 days. Morphologically similar organisms isolated from mouse sarcoma 180 were grown in Dubos' and Alexander-Jackson's liquid media. The granules are characteristically smaller, but otherwise present the same general features as those derived from other groups. More recently, successful isolation of another similar strain of these organisms from a primary lung cancer in a mouse has been accomplished.

MORPHOLOGIC AND CULTURAL CHARACTERISTICS. It has been difficult to classify these organisms on the basis of the usual criteria, that is, morphologic, staining, cultural, and physiologic characteristics. This difficulty is due in large measure to the extremely slow rate and sparsity of growth obtained in the best of culture media. When observed in tissues the organisms are present in both acid-fast and non-acid-fast forms as granules, short rods, and globoid bodies. In culture media minute acid-fast granules predominate in young cultures. As the culture ages, these granules increase in size until they become as large as staphylococci, or even larger, some apparently developing into small acid-fast rod-like forms. Subsequently, the larger granules and rod-like forms appear to pass through the acid-fast state to one which retains the Ziehl-Neelsen fuchsin only when decolorized with ethyl alcohol alone. Ultimately they may lose this acid-fast quality altogether and stain with ordinary dyes. Certain of these rod-like forms contain acid-fast granules while the remaining structures fail to hold the fuchsin stain. This is particularly true of old cultures which contain many of these irregularly staining forms. True branching filaments have not been observed, although unusual forms are often found in old cultures. The granules are gram-positive in character, while the matrix material described below stains poorly.

The variation in size of the acid-fast granules from the lower limits of

the light microscope up to a micron or more in diameter has been a matter of great interest. Because of the gradation in size, it was suspected that granules beyond the range of the light microscope might be found. As will be shown, such granules are commonly observed in electron microscope studies. To date we have filtered 10 cultures: 1 sarcoma of human origin, 1 bladder carcinoma, 3 human mammary cancers, as well as 2 cultures from Rous chicken sarcoma, 1 from mouse sarcoma 180 and 1 case of spontaneous breast cancer in a dog. No difficulty was experienced in recovering the cultures from filtrates passing Chamberland L3 and Seitz filters.

These organisms have been grown on the egg media of Petragnani and of Lowenstein, in Dubos' medium, brain heart infusion plus glycerine and various modifications of the latter. A medium devised by Alexander-Jackson for cultivating mycobacteria has given somewhat better growth. Even in blood and serum the growth is slow and never heavy. Because of the characteristic slow growth, cultures of these organisms are grown in tightly stoppered tubes. One of the most typical growth characteristics is the precipitate which forms in cultures in a liquid medium. It resembles a mass of minute granules held together by fine filaments. As growth becomes more obvious the original mass breaks on slight disturbance, forming other small flocculent growths. The supernatant liquid remains clear. Stained slides from young cultures also show this pattern of granules held together by a fine lightly staining network or matrix[9]. This pattern also appears in the electron microscopic studies. On heart-brain agar slants, growth appears after 14 days as very small discrete colonies. In shake cultures of the same media, the growth is confined to the uppermost portion of the tube, but surface colonies are not observed. The organisms appear to be favored by a slightly reduced oxygen tension. An increased carbon dioxide tension did not improve growth in liquid, shake, or agar slant cultures.

The growth characteristics of the organisms isolated directly from the blood of advanced cancer patients in Dubos' liquid albumin medium are most interesting. The organisms appear to be embedded in irregular strands of collagen-like material. The minute acid-fast forms are present as well as aggregates of larger globoid bodies in this matrix. These aggregates strongly resemble groups of mast cells which have disintegrated and released their granules. These forms stain with toluidin-blue as do mast cell granules. It seems possible that certain granules which have not infrequently been observed within diseased tissues and which frequently have been called mast cell granules, may be globoidal forms of a microorganism of this type. Similar forms have been reported by Diller[4], Cuttino[3], and Grigoraki[6]. The recently published work of Gerlach[5] includes many illustrations corresponding to our own observations.

Electron Microscopic Studies. Many of the smaller bodies were beyond the range of the light microscope and were filtrable on the basis of size alone. Repeated electron microscopic studies were made before and after passage through mice of pure strains isolated from patients with cancer and scleroderma, and from chickens infected with the Rous virus. Preparations were made from both solid and liquid media. The preparation of specimens from the cultures on egg media involved the following procedure: 1) The complete removal of a single colony from the surface without removing any of the medium; 2) the spreading of the colony between mic-

roscope slides; 3) the immediate suspension of the organisms in a few drops of distilled water dropped on one of the slides; 4) the evaporation of a small drop of the suspension on a mounted collodion membrane.

The specimens from the cultures in liquid Dubos' medium were prepared in the following manner: 1) Centrifugation of approximately 1 ml. of culture for 5 minutes at 5000 r.p.m. in a small angle centrifuge; 2) resuspending in 1 ml. of distilled water after pipetting off the supernatant liquid; 3) evaporation of a small drop on a mounted collodion membrane; 4) washing the mount after drying with a few drops of distilled water if examination in electron microscope showed the presence of salt.

The growths appeared to consist of 2 distinctly different but intimately related structures. There was a matrix of fine material consisting of small granules 20 mμ to 70 mμ in diameter connected by a mesh of very fine fibers approximately 1 to 2 mμ in diameter. Interspersed in this matrix and physically held in it were more or less spherical and dense granules or glob-

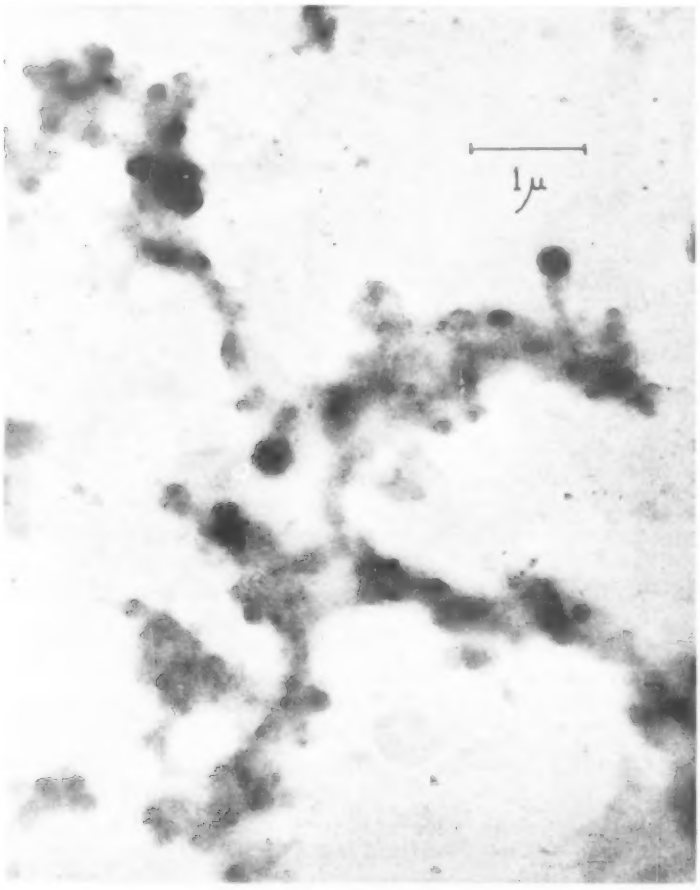


FIG. 1.—Electron microscope photograph of culture from mouse sarcoma 180. Note globoid bodies embedded in matrix of small granules and connecting fibers (X 31,000).

ules. These globules had widely varying diameters ranging from 1.5 μ to 0.2 μ. Their distribution in the matrix was quite characteristic in that individual particles or large closely linked groups were rarely seen. On the other hand, small loose groups of from 2 to 7 globules and "budding" forms consisting of 2 particles of different diameters in close association were very common. The spacing and size distributions of the globules, the presence of "budding" forms, and the lightly staining but unresolved matrix provide the basis for identifying the organism observed with the light microscope in tissue section with those observed in the cultures by the electron microscope.

Pathogenicity Studies. Pathogenicity studies of 4 separate strains of these organisms were undertaken with *a*) 2 isolates made directly from human cancer* patients—the 1st from tissue from a breast cancer; the 2nd from the blood of a patient with advanced sarcoma of the hand; *b*) an isolate from a Rous chicken sarcoma; and *c*) an isolate obtained from the 2nd mouse passage of a scleroderma strain derived from a patient's blood. All inoculations were made as a heavy suspension of the culture in liquid media. Twelve mice were inoculated intraperitoneally with 0.2 ml. each of the respective cultures. The 12 control mice received 0.2 ml. of medium. From time to time as the infected mice began to show evidence of disease, they were sacrificed, along with an equal number of controls. Suspensions were made of tissues or blood and then subinoculated into another series. Cultures were routinely made, whenever possible, from all animals sacrificed or dying spontaneously. However, some of the infected animals died and were eaten by their cage mates during the night before cultures could be obtained. A total of 138 mice of unselected strains were used, of which 60 were controls. All 60 controls were examined by post mortem section and culture. Of the infected animals, 35 were similarly cultured and sectioned.

In discussing the pathologic changes induced experimentally in various animals by these organisms, it is well to keep in mind that the problems posed by these studies bear little, if any, relationship to the well established species specificity of such tumors as Rous sarcoma or sarcoma 180 of the mouse. We fully recognize, as do all investigators in the experimental field, that the Rous chicken sarcoma is caused by a virus or filtrable agent and that the tumor cannot be transmitted successfully to other animal species. Similarly, sarcoma 180 must be transplanted into another mouse and ordinarily cannot be induced in any other experimental animal.

The pathologic changes described in this paper are lesions produced *not* by the cells of Rous sarcoma nor by the cells of mouse sarcoma 180, but by any of the several strains of organisms recovered from these and other tumors, including cases of human malignancy. An outstanding feature of interest is that these organisms are apparently not species specific inasmuch as organisms recovered from Rous sarcoma can induce essentially the same response in mice, rats, guinea pigs and rabbits as well as in chickens. Similarly the strain of organisms recovered from mouse sarcoma 180 (or for that matter from any other neoplasm from which this type of organism has been obtained) will produce characteristic lesions when injected experimentally into any of the common laboratory animals.

PATHOLOGIC CHANGES. The lesions (Fig. 2) may be described in general as pseudo-caseous, rather widely distributed but tending to occur prin-

* Since the breast cancer strain was thought to be attenuated after 18 months of artificial cultures, a freshly isolated culture from the blood of a young boy with advanced sarcoma of the hand was also studied. Both the original breast isolate as well as the one more recently obtained from the sarcoma were inoculated into guinea pigs, both intraperitoneally and subcutaneously. From this experiment, there was little evidence of attenuation of the original breast cancer strain, although the more recently isolated tissue produced more marked proliferative changes in the experimental animals' tissues.

cipally in the liver, kidneys, and lungs, although at times involvement of the heart, spleen, adrenal, stomach, and lymph nodes have been noted. In at least 1 instance rather diffuse infiltration of the omental fat was observed.

It is perhaps of significance to note that with each successive animal transplant, the time factor in the development of these lesions tends to be reduced until, in the 3rd or 4th animal passage, it becomes a matter of 10 days to 2 weeks rather than weeks or months. It is also interesting to note that litter mice from infected mothers frequently show identically appearing gross lesions, which as a rule develop only after several weeks. The litters of these mice are usually small in number and stunted.

GROSS PATHOLOGY. A description of any one of these lesions will serve as a pattern for them all, although considerable variation exists in respect to the severity of the reaction, the size and number of the lesions and their distribution. These differences vary, not only with different strains of these organisms but also with individual animals inoculated with the same strain. The typical lesion varies from 2 or 3 mm. up to 8 or 10 mm. in diameter. The majority of these lesions are discrete, but in some instances, particularly in the liver, a number of these small foci coalesce to form a larger conglomerate lesion, occupying perhaps a quarter to a third of the volume of the organ. This is less noticeable in the lungs and kidneys, although even here the same tendency for the lesions to coalesce is observed. No such conglomerate nodules have been noted in the other viscera.

The individual lesion tends to be rather yellowish-gray in color with scattered areas of hemorrhage at times. The lesions are rather nodular in character, and frequently elevated 1 to 2 mm. above the surface of the organ, especially in the liver. In other instances they are buried centrally in the particular organ involved. While these nodular foci are fairly well localized, yet at times they can be seen extending into the surrounding organ and tissues. On section of such a lesion, it appears as a yellow-gray, somewhat firm, elastic caseous area of necrosis, not unlike in some respects that seen in association with tubercle or even miliary gumma formation. As a rule, however, the color is much more yellow than is customarily noted in these

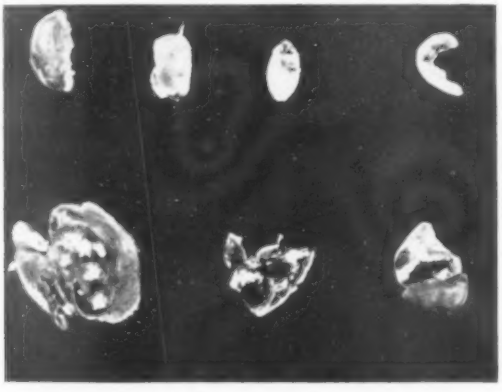


FIG. 2.—Gross lesions involving particularly the liver, lungs and kidney of a mouse inoculated with a culture isolated from the Rous chicken sarcoma.

2 other conditions. Neither actual pus formation nor true liquefaction necrosis is found, thus readily differentiating these lesions from any of the ordinary pyogenic infections.

Microscopic Pathology. These necrotic foci reveal a basic common pattern, but with marked variation of the picture. The lesion consists of a central area of necrosis made up of masses of organisms occurring almost in colony formation. In hematoxylin and eosin stained preparations, under oil immersion, this necrotic mass can be resolved into a network of eosinophilic staining material in which are embedded tiny globoid bodies tending to stain with the hematoxylin. These coccoid bodies occur sometimes singly, sometimes almost as chains, and at other times in clumps. It also is possible at times to observe coccobacillary forms containing these granules. Toward the periphery of these

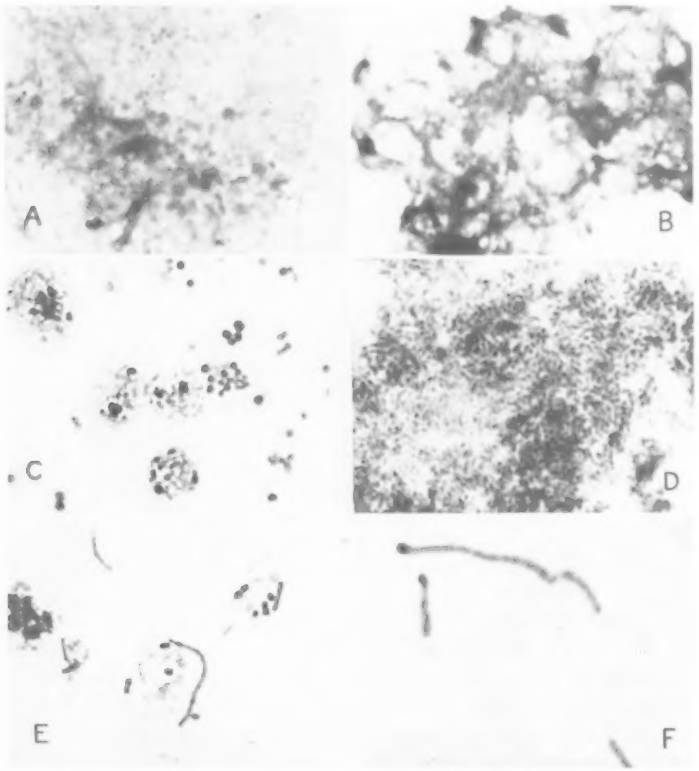


Fig. 3.—(a and b) Plasmodial mass with numerous minute bodies, some of which are distinctly acid-fast and lie in the center of an apparently clear area (cf. (a)). From 2nd passage of a blood culture grown from a human cancer patient "L" in Alexander-Jackson and Difco brain heart liquid media (X 2000). (c) Larger globoidal bodies in groups, some of which are held within a meshwork enclosed by a membrane-like envelope 3 to 4μ in diameter. From 2nd passage of a blood culture "K" grown from a human cancer patient in Alexander-Jackson and Difco brain heart liquid media (X 2000). (d) Culture from 3rd mouse passage of strain isolated from human mammary cancer. Note the predominantly coccobacillary form of the organism (X 2500). (e and f) Filamentous and rod-like forms observed in an autopsy culture obtained from the bone marrow of a leukemia patient, and grown in Alexander-Jackson and Difco brain heart liquid (X 2500 and 4500 respectively).

colony-like masses, the individual organisms can be much more readily distinguished. Here, marked variation in their size and shape is particularly evident with the appearance of short rod-like forms. With Ziehl-Neelsen stained preparations both acidophilic and non-acidophilic forms occur, the acidophilic predominating.*

Surrounding this mass of organisms is a zone of cellular infiltration consisting almost entirely of mononuclear cells of various types, with lymphocytes greatly outnumbering the other types. Large mononuclear phagocytes laden with organisms in both granule and coccobacillary forms are prominent, while plasma cells and other mononuclear cells, difficult if not impossible to identify, are also present in relatively large numbers. Many of these cells undergo degeneration, and free forms of the organism are found in the interstitial spaces. The presence of these organisms likewise appears to stimulate the increase of fibroblasts, and both young and older forms of these cells are regularly found. There is some suggestion that this microbial invasion tends to involve particularly the collagenous tissues. This is particularly seen in the kidney and lung.

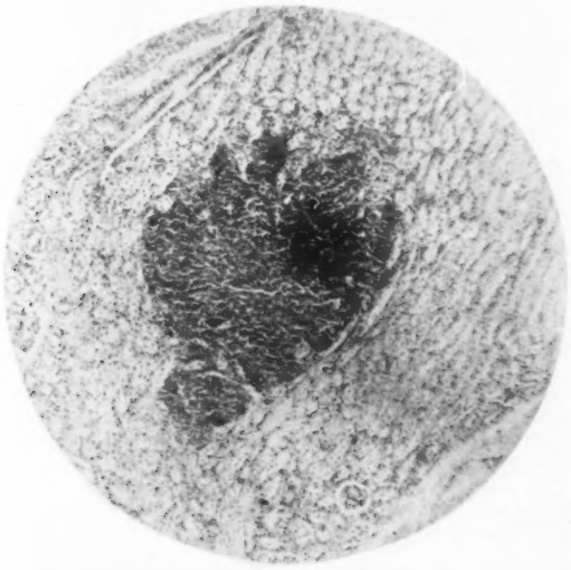


FIG. 4.—Kidney of mouse. Low power magnification (X 100) of a lesion resulting from spontaneous inoculation with a strain of organisms from a Rous sarcoma. Note caseous necrosis and finger-like extension of the lesion into surrounding tissues.

* Careful histologic study of tissues from the 35 experimentally inoculated and 60 control mice mentioned earlier was carried out, sections being stained by routine hematoxylin eosin and Ziehl-Neelsen techniques. All the infected animals showed the presence of the organisms in profusion, while in no instance was it possible to demonstrate them in the control tissues. Similar studies of tissues from guinea pigs inoculated with cultures of this organism gave confirmatory results. Histologic examination of human tumor material taken from over 50 neoplasms of various types and stained with carbol-fuchsin, regularly showed the presence of similar appearing acid-fast and non-acid-fast granules and rods. No organisms could be demonstrated in tissues from normal autopsy cases.

where the lesions appear principally in the intertubular area and in the alveolar walls, breaking through into the alveoli themselves. This feature is less apparent in the liver, although the fibrous tissue of the portal area is commonly involved.

Comment has been made upon the variations in the basic pattern of this lesion. Nowhere is this more evident than in the animals inoculated with the strains of organisms recovered from a typical Rous sarcoma. The basic lesion still is essentially of a chronic granulomatous nature. The development of no lesion actually resembling the tumor of origin has been observed. Likewise, histologic study of tissues from guinea pigs inoculated experimentally with the most recent human sarcoma isolate show striking proliferative changes of the fibrous tissue (Fig. 6).

Another feature is the not infrequent tendency for necrotic changes to involve blood vessels, both arteries and veins, but particularly the latter. Striking degenerative changes of the connective tissue of the vessel wall are observed, with proliferative changes involving the lining endothelium. In a few instances, where the animal has survived for a sufficient period of time, thrombosis with recanalization of the vessel lumen has occurred. Occasionally, subintimal clumps of mononuclears, indistinguishable from those found in the periphery of the focal lesions, are encountered. As these vessels are usually near the area of caseous necrosis, they may merely represent an extension of the process from vessels passing through the major lesion. At times, however, they have no such apparent relationship and the possibility of the organisms acting directly on the vascular endothelium and vessel wall cannot be excluded.

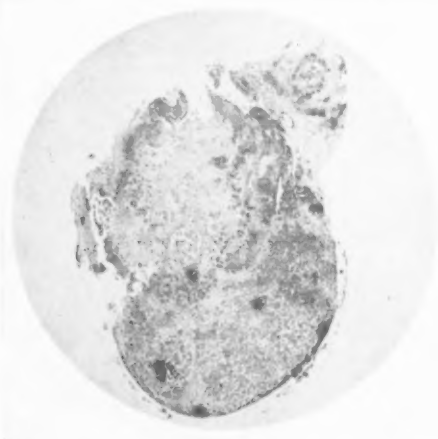


FIG. 5.—Lung of mouse. Microtessar photograph (X 16) of section of lung showing multiple extensive caseous-like areas resulting from inoculation with a strain of organisms derived from a case of breast cancer. Note the colony-like mass of organisms round the periphery of the lesion.

Summary. 1. A group of morphologically similar microorganisms, varying considerably in size and shape, has been observed in and isolated from various proliferative diseases of man and animals, including scleroderma, human cancer, Rous sarcoma and mouse sarcoma 180.

2. These organisms, which appear primarily as small acid-fast granules in young cultures and which tend to become non-acid-fast in the larger forms present in old cultures, may exhibit a number of types, such as: *a*) minute filterable granules beyond the limits of visibility of the light microscope; *b*) larger granules approximately the size of ordinary cocci, readily seen with the light microscope; *c*) later globoidal forms; *d*) rod-like forms with irregular staining; and *e*) occasionally globoidal forms which appear to undergo polar budding.

3. We have not been able thus far to recover the organism from 17 normal controls nor from patients suffering from any other condition.

4. Cultures inoculated into experimental animals produce a characteristic pseudocaseous lesion. Huge numbers of lymphocytes are called out as part of the response.

5. Final classification of these organisms cannot be made at this time. That they may represent isolated instances of a group of biologically related organisms capable of causing proliferation in the host is not overlooked.

6. Whether these organisms are of primary or secondary significance in the development of malignant disease remains to be established.

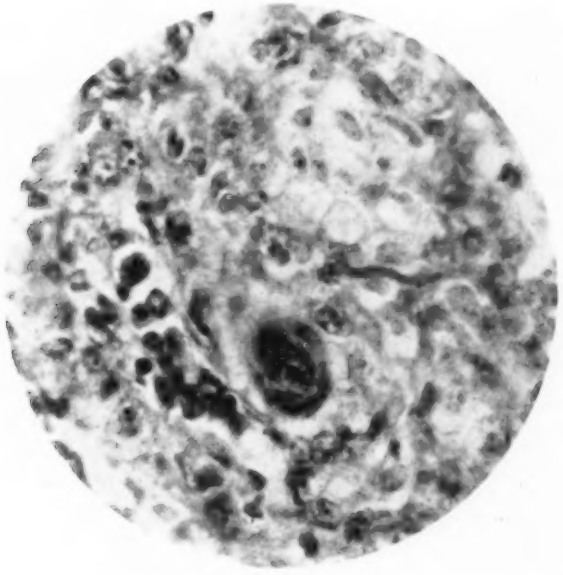


Fig. 6.–Guinea pig lung inoculated with an organism from a case of human fibrosarcoma (X 1000). Note large cell with abnormal chromatin arrangement.

Grateful acknowledgement is made to Dr. Royal A. Schaaf, Chief of Staff of the Presbyterian Hospital, Newark, for his invaluable cooperation, to Mr. Paul Little, Lederle Laboratories, for the Rous sarcoma material, to Dr. Sonia Buckley, Sloan-Kettering Institute, for the sarcoma 180 mice, and to Dr. S. A. Goldberg, pathologist, Presbyterian Hospital, Newark, for his kind assistance and advice.

REFERENCES

1. **Alexander-Jackson, E.**: Ann. N. Y. Acad. Sci., **46**, 127, 1945.
2. Idem: Science, **101**, 563, 1945.
3. **Cuttino, J. T.**, and **McCabe, A. M.**: Am. J. Path., **25**, 1, 1949.
4. **Diller, I. C.**: Anat. Rec., **105**, 24, 1949.
5. **Gerlach, F.**: Krebs und obligater Pilzparasitismus, Wien, Urban & Schwartzenberg, 1948.
6. **Grigoraki, L.**: Edit. Lefrancois, Paris, 1944. From J. Alexander, Colloid Chemistry, Theoretical and Applied, Vol. 7-8, New York, Reinhold. In press.
7. **Wuerthele-Caspé, V.**: J. Am. Med. Women's Assn., **4**, 135, 1949.
8. Idem: J. Med. Soc. New Jersey, **44**, 52, 1947.
9. **Wuerthele-Caspé, V., Allen, R. M.**, and **Rose, S. J.**: Bull. N. Y. Micro. Soc. **2**, 1, 1948.
10. **Wuerthele-Caspé V., Brodkin, E.**, and **Mermod, C.**: J. Med. Soc. New Jersey, **44**, 256, 1947.

THE LOCUS OF THE PERIPHERAL VASODILATOR ACTION OF ETHYL ALCOHOL, TETRAETHYLAMMONIUM AND PRISCOLINE

By Lawrence L. Siems, M.D.

AND

H. S. Rottenstein, M.D.

PHILADELPHIA, PENNSYLVANIA

(From the Department of Pharmacology, School of Medicine, University of Pennsylvania, and the Peripheral Vascular Section of the Robinette Foundation of the Department of Medicine of the University of Pennsylvania)

In recent years there has been a mounting interest in improving the therapeutic use of drugs effective in increasing the blood flow to the extremities. The interpretation of observed effects and the logical basis for clinical usage of these drugs depend in part upon a knowledge of their sites of action—whether they act directly on the walls of vessels, indirectly through vasomotor nerve influence, or by some combination of both. It has been difficult to acquire unambiguous evidence of the sites of their action; hence, it was the purpose of the present investigation to obtain evidence of the site of action of 3 vasodilator drugs now receiving extensive clinical trial: ethyl alcohol, tetraëthylammonium chloride (hereafter T.E.A.) and Priscoline (2 benzyl-4, 5-imadazoline hydrochloride).

The usual method of testing a substance for local vasodilator action has been to inject intra-arterially doses which would be ineffective if introduced into the general circulation. Unfortunately, in order to observe a local effect it may be necessary to expose the tissue to such a concentration of the drug, or to maintain the exposure for such a length of time, that the required doses will produce centrally mediated (vasomotor nerve) effects. Conversely, centrally mediated actions of a drug with powerful local dilator action may be masked by the local effect. Therefore, results obtained with intra-arterial injection are often inconclusive. For this investigation a new method has been devised which obviates these difficulties.

Methods. All experiments were conducted on morphine-pentobarbital anesthetized dogs in a room which was constantly maintained at 30° ± 1°C. This temperature was selected to prevent extensive loss of body heat from the anesthetized animals[11].

On the left side of one dog and the right of another the following structures were isolated: the common carotid artery; the external jugular vein; the femoral artery, vein, and nerve; and the sciatic nerve. A wire ligature was passed about each prepared thigh beneath the intact vessels and nerves and tightly secured. Each dog was given 5 cc. of heparin intravenously. Cannulae were inserted into the femoral vessels pointing distally and into the neck vessels pointing centrally (Fig. 1). Using "Tygon" tubing the common carotid of 1 animal (Dog A) was connected to a motor-driven DeBakey pump with shunt attachments; and thence to a modified bubble flowmeter[5]. The "downstream" end of the meter was used to supply blood to the femoral artery of the other animal (Dog B). Tubing was then used to connect the femoral vein of Dog B to the external jugular vein of Dog A. An identical "circuit" was established between the neck vessels of Dog B and the leg vessels of Dog A. Thus, in each experiment, each animal

* Expenses were defrayed in part by grants from the Life Insurance Medical Research Fund, the U. S. Public Health Service, and from Smith, Kline and French Laboratories.

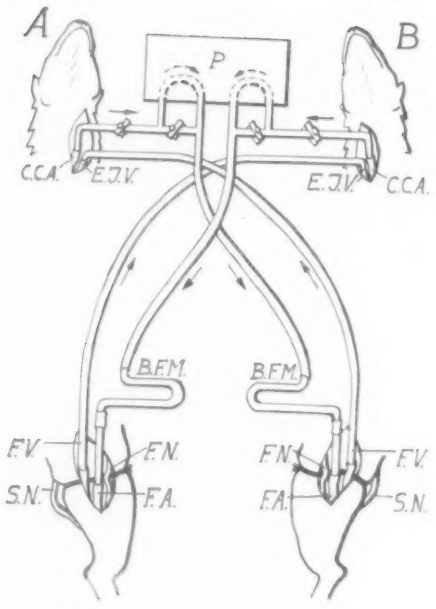


FIG. 1.—Schematic Presentation of Perfusion Circuits

B.F.M. = bubble flowmeter
C.C.A. = common carotid artery
E.J.V. = external jugular vein
F.A. = femoral artery
F.N. = femoral vein
F.V. = femoral nerve
P. = DeBakey pump
S.N. = sciatic nerve

supplied circulating blood to one hind limb of his fellow. The placement of the ligature prevented appreciable mixing of the circulations as suggested by Green and co-workers[6]. This was further substantiated by testing for the appearance of Dye T1824, in the blood of one animal after injecting it into the other.

Each leg was perfused at a constant pressure of 100 mm. Hg. by means of adjustments of the shunts attached to the pump. The shunts also permitted independent regulation of flow in the 2 circuits. By eliminating variations in blood pressure it was possible to interpret changes in peripheral resistance directly from blood flow changes. Blood pressure was measured by means of air damped mercury manometers attached to the "bubble traps" of each bubble flowmeter. Although the manometers measured lateral pressures, the cross-sectional areas of the "bubble traps" were sufficiently great that even at the extremes of the flow range the measured pressures were only 1 to 3 mm. less than the "head on" pressures.

A slow-drip intravenous infusion of a gelatin solution (a plasma substitute) was given each animal during the experiment to replace blood loss resulting from slow incisional oozing after heparinization.**

With the preparation complete, after waiting 10 to 20 minutes for the subsidence of reactive hyperemia, the flow in each perfused limb was measured for 30 minutes, taking readings every 5 minutes. At this time the drug under investigation was injected intravenously into one of the animals and flow readings continued for variable periods (always at least one half hour) depending on the stability of the preparation. It is apparent that the injected drug did not reach the perfused leg of the animal into which it was injected; hence, any resulting vasodilatation must have been mediated through the intact nerve supply. Conversely, the perfused leg of the other animal received drug-containing blood, and any effects must have been obtained locally since the drug did not reach the systemic circulation of this animal.

Priscoline was given in doses of 1 mg. per kg. by rapid injection. Alcohol was given in a fixed dose of 5 cc. of a 95% solution, diluted to 10 cc. with 0.9% saline, and injected over a 15 minute period by means of a motor-driven constant injection unit. T. E. A. was given in doses of 5 mg. per kg. injected over a period of 5 minutes. Preliminary experiments showed that 10 mg. or more per kg. frequently produced shock, whereas 2 mg. per kg. could not be depended upon to produce vasodilatation. Indeed, rapid injection of 5 mg. per kg. might produce shock.

In analyzing the results, the 30 minute reading was chosen as the base line, this being necessary because invariably the blood flow trend was downward. The positive response to the drugs varied considerably for every animal, being most pronounced toward the end of the experiments when the flows in some animals tended to return to control levels and in others remained at very high levels. For this reason the readings taken 15 minutes after completion of drug injection were chosen as the check points for statistical analysis, since drug effects were always present although frequently not maximal. The drugs were known peripheral vasodilators. Such points of comparison were necessary only to demonstrate the statistical validity of conclusions. It is evident from the

** Supplied through the courtesy of Knox Gelatin Company.

graphs of averaged results that the effects of drug injection were quite definite. Significance was determined by the method of paired comparisons for the difference between the base line and post injection check point.

Results. *Priscoline.* Fig. 2 and the Table present the average blood flow changes observed after the injection of this drug in 7 experiments (14 animals). It is apparent that significant flow increase occurred in only those limbs receiving drug-containing blood.

Alcohol. Fig. 3 and the Table show

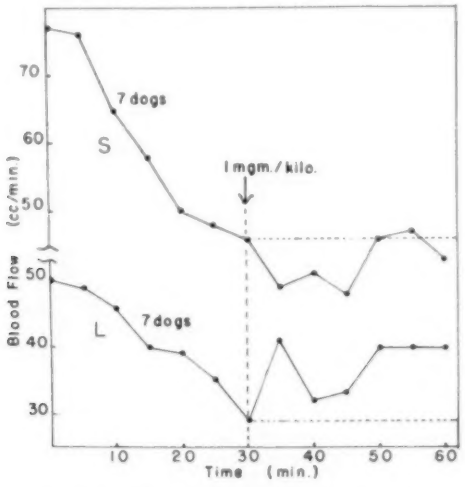


FIG. 2.–Effect on Femoral Blood Flow of the Injection of Priscoline
L. = legs receiving drug containing blood
S. = legs of animals receiving drug systemically
Horizontal dashed lines = 30 minute base line
Vertical dashed lines = point of drug injection

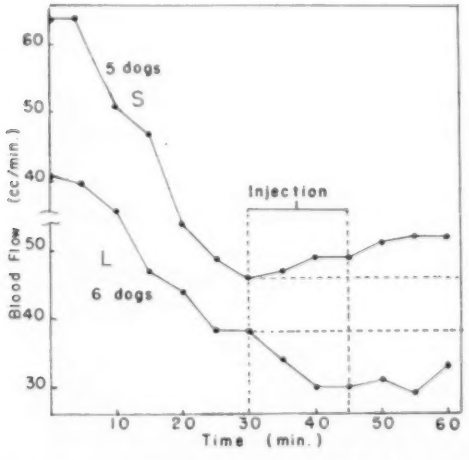


FIG. 3–Effect on Femoral Blood Flow of the Injection of Ethyl Alcohol
L. and S. as in FIG. 2.
Vertical dashed lines = period of drug injection

the results of 6 experiments (11 animals) with alcohol. Increased flow was seen in only the limbs of animals having received the drug systemically, and hence must have been mediated through the nerve supply. Although the attempt was made to follow all animals of this group for a longer period than 1 hour, mishaps forcing the premature conclusion of 2 experiments made the later data unsuitable for presentation. Those data seem to indicate, however, that the 60 minute readings did not represent the height of alcohol effect.

T.E.A. Fig. 4 and the Table show the results in 5 experiments (10 animals) of this drug. Since flows increased significantly in both groups of limbs, it is obvious that the drug produced both local and nerve mediated vasodilatation.

TABLE 1.—RESULTS OF DRUG INJECTION ON BLOOD FLOW TO THE HIND LIMB

			Blood Flow cc./Minute			
Drug	*No. of Animals*	*Type of Perfusion*	*Control, 30 min. reading*	*15 min. after completion injection*	*Increase*	*t ratio*
Priscoline	7	S	46	38	−8	1.6
	7	L	29	33	+4	3.0*
Alcohol	5	S	26	32	+6	3.3*
	6	L	38	33	−5	.82
T. E. A.	5	S	26	34	+8	5.2**
	5	L	24	44	+20	3.1*

S indicates that the animals received the drug systemically but that it did not reach the limb studied.
L indicates that the limbs studied were perfused with drug containing blood but the drug did not reach the systemic circulation of the animals.
* indicates significance at greater than the 5% level.
** indicates significance at greater than the 1% level.

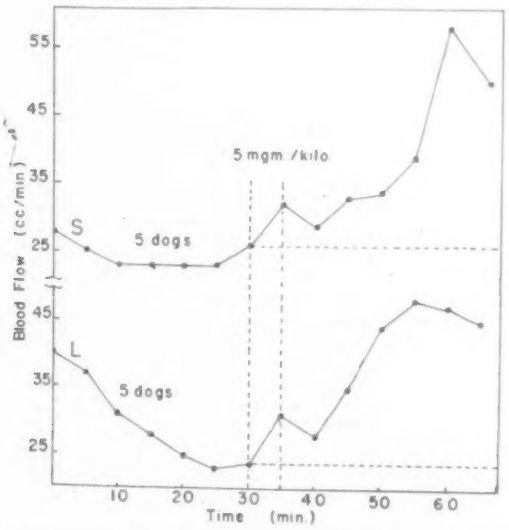


FIG. 4.—Effect on Femoral Blood Flow of the Injection of T.E.A.
L. and S. as in FIG. 3

Discussion. Since this approach to the problem of peripheral vasodilator drug action is new, a short discussion of the method is in order. It is designed only to differentiate between local effects and effects mediated through the vasomotor nerve outflow. It is not well suited for evaluating relative potency or for further localizing actions of drugs. It has the advantage of permitting a clear cut separation of effects; and, because of the constant perfusion pressure, allows direct interpretation of change in peripheral resistance through changes in blood flow. The principal disadvantage is the complexity of the preparation. As a result the many extraneous accidents which may occur make the method formidable if used as an occasional procedure.

Priscoline has received clinical trial as a peripheral vasodilator because of its adrenergic blocking action and because it may produce vasodilatation in the absence of sympathetic innervation[10]. It has been suggested that the drug also may produce vasodilatation through an effect on the central nervous system[8]. The present study clearly demonstrates the local action of Priscoline. No evidence of an indirect influence through action of vasomotor nerves was seen. Although it is possible that such an effect might be obtained with very high dosage, the dosage in the present experiments was somewhat higher than the usual effective dose used in man and animals[3]. It is unlikely that the use of higher dosage would ever be considered practical.

Alcohol has long been used medically for its peripheral vasodilator properties, especially with regard to cutaneous vessels. It is generally thought that this action is due to its influence on the vasomotor centers[12]. The present study confirms the belief that the vasodilator action of alcohol is mediated through vasomotor nerve outflow.

T.E.A. has been of interest because of its ability to block ganglionic transmission of autonomic outflow[2]. It has marked vasodilator action[2,4,9]. Although it has been presumed that vasodilatation is the result of blocking vasoconstrictor nerve influence at ganglia, the results of this study indicate that there is in addition a locally effective vasodilator component which is at least equal in magnitude to the indirect action. The mechanism of this local action cannot be explained on the basis of the known properties of T.E.A. except on the hypothesis that there are autonomic ganglia within the hind limb of the dog. Since there is no anatomic evidence for this assumption, it is likely that the effect is due to a heretofore unsuspected local action of T.E.A.

It should be emphasized that the actions of these drugs on the hind extremity do not imply widespread effects throughout the body. Horwitz and co-workers[7] in human experiments showed that Priscoline and alcohol increase human digital cutaneous blood flow but not cardiac output. Therefore, the effects were certainly not generalized. In contradistinction they found that effective doses of T.E.A. causing increases in digital cutaneous flow did increase cardiac output, and the effects were, therefore, more generalized. Furthermore, Abramson[1] cites evidence that flow responses to various stimuli may be quite dissimilar in various portions of an extremity. Our experiments deal with the limb as a whole, and it is not possible to state which portion or portions of the extremities in the present study were affected by the vasodilator drugs.

Summary and Conclusions. 1. A new method is presented which permits direct differentiation between vasomotor-nerve-mediated and local peripheral vasodilator drug actions.

2. Priscoline when given in the usual

effective amounts had only local peripheral dilator properties.

3. Alcohol acted indirectly through the vasomotor nerve supply to the limb.

4. Tetraëthylammonium chloride had both vasomotor-nerve-mediated and previously unsuspected local peripheral vasodilator properties. This latter effect cannot be explained by the known mechanisms of action of the drug.

REFERENCES

1. **Abramson, D. L.:** Vascular Responses of the Extremities in Health and Disease, p. 163, Univ. of Chicago Press, 1944.
2. **Acheson, G. H.,** and **Moe, G. K.:** J. Pharmacol. and Exp. Therap., **87**, 220, 1946.
3. **Ahlquist, R. P., Huggins, R. A.,** and **Woodbury, R. A.:** Ibid., **89**, 271, 1947.
4. **Brown, G. E., Wood, E. H.:** Ibid. **93**, 10, 1948.
5. **Dumke, R. R.,** and **Schmidt, C. F.:** Am. J. Physiol., **138**, 421, 1942.
6. **Green, H. D., Cosby, R. S.,** and **Radzow, K. H.:** Ibid., **140**, 726, 1944.
7. **Horwitz, O., Montgomery, H., Longaker, E. D.,** and **Sayen, A.:** Am. J. Med. Sci., **218**, 669, 1949.
8. **Meier, R.,** and **Miller, R.:** Schweiz. med. Woch., **69**, 1271, 1939.
9. **Malton, D., Hoobler, S. W., Ballantine, H. T., Nelight, R. B., Cohen, S.,** and **Lyons, R. H.:** Fed. Proc., **6**, 353, 1947.
10. **Nickerson, M.:** J. Pharmacol. and Exp. Ther. (Pharmacol. Rev.), **95**, 27, 1949.
11. **Siems, L. L.,** and **Kosman, A. J.:** Am. J. Physiol., **156**, 185, 1949.
12. **Sollmann, T.,** A Manual of Pharmacology, p. 612, Philadelphia, W. B. Saunders, 1948.

THE OCCURRENCE OF RENAL INSUFFICIENCY IN SUBACUTE BACTERIAL ENDOCARDITIS

By HERMAN VILLARREAL, M.D.*
ROCKEFELLER FOUNDATION FELLOW
AND
LEON SOKOLOFF, M.D.
ASSISTANT PROFESSOR OF PATHOLOGY
NEW YORK, NEW YORK

(From the Department of Pathology, New York University College of Medicine and Bellevue Hospital)

ALTHOUGH renal lesions are a well-known feature of subacute bacterial endocarditis, it is not so generally recognized that these may lead to renal insufficiency. Aside from infarction, embolic glomerulonephritis is the characteristic lesion found in the majority of cases. In addition, the occurrence of banal types of glomerulonephritis has been reported several times to be of high incidence.

Embolic glomerulonephritis, as described by Baehr[2], characterized by: 1) involvement of one or more loops of a variable number of glomeruli; 2) absence of any visible lesion in the uninvolved glomeruli and in uninvolved portions of the affected glomeruli; and 3) coexistence, in most cases, of glomerular lesions in all stages of development. Baehr[3] observed that, except for slight albuminuria and hematuria, symptoms indicating a serious disturbance in renal function, do not appear. In the original description of the lesions, Löhlein[14] also stated that edema and uremia are very infrequent; nevertheless he considered it likely that if the process was sufficiently extensive, renal insufficiency might result. In a recent report, Boyarsky, Burnett and Barker[7] were able to collect only 16 instances from the literature in which uremia due to embolic glomerulonephritis complicated subacute bacterial endocarditis. Bell[5] found the kidneys of 5 of 108 cases of subacute bacterial endocarditis to have embolic lesions so extensive as to give "ample anatomical evidence of renal insufficiency". Unfortunately corresponding clinical data were not presented; the relatively good renal function in face of severe anatomical lesions has been stressed by the previously cited investigators. Christian[9] found no instances of definite uremia in 61 cases of subacute *Strep. viridans* endocarditis. Fishberg[12] quotes Libman as not having seen uremia in any of 800 cases of subacute bacterial endocarditis that were not in the bacteria-free stage. Elsewhere, however, Libman and Friedberg[13] reported some degree of renal insufficiency in one-third of cases in the bacteria-free stage.

This discrepancy is explained by Baehr[3,4] by the finding of "diffuse" nephritis in those cases in which uremia supervenes (9 of 77 cases). Most American textbooks of pathology do not describe the coexistence of these entities. Boyd[8] states that it is "rather uncommon". Nevertheless Bell found 10 instances of well developed acute and chronic glomerulonephritis

* Present Address: Instituto Nacional de Cardiologia, Mexico, D. F.

in 108 cases of subacute bacterial endocarditis. In the previously cited report from the Johns Hopkins Hospital[7], glomerulonephritis with marked azotemia was found in 4 of 83 autopsied cases. Libman associated this form of renal disease with a state of hypergy to the *Strep. viridans* that develops in the so-called bacteria-free stage of the disease.

It has been our impression that renal insufficiency is not as infrequent a sequel of subacute bacterial endocarditis as some of these reports would indicate. The existence of a "renal form" of the endocarditis, that is, one in which symptoms of nephritis dominate the clinical picture, is apparently well recognized on the European continent[10,15].

Material and Methods. The clinical records, necropsy protocols and histologic sections of 100 necropsies on individuals dying of subacute bacterial endocarditis at Bellevue Hospital between 1937 and 1949 were reviewed. In most instances, 1 to 3 sections, and usually 2, of kidney were available for study. These were routinely stained with hematoxylin and eosin, and special stains (azo-carmine) were infrequent. Blood cultures had been performed at least once during life in 63 cases; from these, the alpha-hemolytic streptococcus was recovered 33 times (52%). Post-mortem cultures of heart's blood, the vegetation or spleen was made in 15 instances.

Uremia is here regarded as "the abnormal elevation of blood urea resulting from intrinsic renal disease"[11]. Not all elevation of concentration of non-protein nitrogen in the blood during the course of subacute bacterial endocarditis is due to uremia. Extrarenal azotemia may in this circumstance be attributed to congestive heart failure and to fever. This was present at least once in 10 of the 61 non-uremic cases in which a non-protein nitrogen determination was made. In 2 of these 10 patients, this value was as high as 69 and 75 mg. per 100 cc. of blood, but usually it was less than 50. In addition, there were 3 cases of chronic pyelonephritis attributable to enlargement of the prostate in which the non-protein nitrogen concentration was considerably elevated. These have not been included with the cases of uremia.

Other evidences of diminished renal function have been utilized when the information was available. These included loss of maximal ability to concentrate the urine or diminished excretion of phenolsulphonephthalein, lowering of the concentration of the calcium in the serum, elevation of the phosphorus and of the blood urea nitrogen and creatinine. Sufficient clinical data were available in most cases to establish the presence of uremia; in some it was possible to make this diagnosis seem only probable, while in others laboratory data were inadequate, although the presence of severe renal lesions suggested that had such tests been made, they might well have revealed the existence of uremia.

In most instances, it has been possible to distinguish the embolic glomerulonephritis from the other forms of glomerulonephritis. In several instances, however, difficulties have been experienced: 1) With advanced healing of the lesions, widespread embolic disease comes to resemble chronic glomerulonephritis very closely.

2) We are inclined to agree with Bell[5], Christian[9], Addis and Oliver[1], rather than with Baehr[2], that the embolic lesions are frequently accompanied by a "glomerulitis" of the uninvolved tufts. This is characterized by an increase of the cellularity of the glomeruli and occasionally of thickening of the basement membrane as well. Usually this is of mild degree, but it may at times be so intense that it resembles acute glomerulonephritis. Indeed, this glomerulitis may occur without embolic glomerular lesions.

3) Embolic glomerulonephritis may coexist with acute or chronic glomerulonephritis[1,16].

4) Occasionally focal areas of glomerular necrosis may occur in glomerulonephritis unaccompanied by bacterial endocarditis (Baehr[3]).

Similar difficulty in classifying a small proportion of cases of widespread renal lesion has been experienced by Baehr[3] and others. An attempt to estimate the degree of involvement in the embolic disease was made by counting 100 glomeruli and the number of these that had the glomerular lesions.

Diminution in the amount of urinary sediment in subacute bacterial endocarditis usually takes place subsequent to therapy with penicillin. It has been suggested that such treatment may possibly lead to healing of the nephritis[6]. For this reason, it was considered necessary to observe the incidence of renal insufficiency in the groups of cases receiving penicillin and those that did not.

Results. In this series of 100 cases embolic glomerular lesions were found

TABLE 1.—CASES OF SUBACUTE BACTERIAL ENDOCARDITIS WITH UREMIA

No.	Blood pressure	Blood concentration non-protein nitrogen mg./100 cc.	Other laboratory data on renal function	Specific gravity of urine	Organism	Renal lesion and kidney weight	Antibiotic therapy	Clinical diagnosis of uremia
1	102/60	30, 50, 75	None	Concentr. test: fixed at 1.010	Blood cult.: Strep. viridans	Embol. glomerulo-neph.; 88% of glom. involved, chiefly recent. 580 gm.	None	+
2	150/78	34, 40, 50, 82, 82	P.S.P. excretion, 2 hrs.: 3%	1.010–1.012 in 9 tests	1 blood cult: no growth. P.M.: no growth	Embol. glomerulo-neph.; 77% of glom. involved, recent and healing. 260 gm.	None	+. Coma with urea frost
3	146/50	53, 50, 64, 75, 125, 180	None	1.007–1.011 in 10 tests	Blood cult.: Strep. viridans	Embol. glomerulo-neph.: 80% of glom. involved, recent and healing. 690 gm.	None	+
4	136/58 180/76	112, 100, 180	Blood creatinine: 3.0 mg./100 cc.	1.013–1.015 in 4 tests	Cults. not made	Embol. glomerulo-neph.; 95% of glom. involved, recent and healed. 330 gm.	None	+
5	196/32–300/0	69, 75. Blood urea N.: 154	Phenol Red excretion test: 8%; urea clearance: 9.6% of normal	1.008–1.018 in 10 tests	Repeated blood cults: no growth	Chr. glomerulo-neph. 380 gm.	None	Implied: chr. and embol. glomerulo-neph.
6	150/75	38, 40, 45, 50, 60, 60, 100	None	1.010 in 2 tests	2 blood cults.: no growth	Embol. glomerulo-neph.; 62% of glom. involved. 620 gm.	None	+. Ac. fibrin. pericard.
7	135/80	120, 180, 224	Serum Ca.: 8.2 mg. Serum P.: 6.7 mg. CO_2 combining capacity 27, 24 vol.%	1.007–1.010 in 4 tests	No cults. made	Embol. glomerulo-neph.; 88% of glom. involved, chiefly healing. 420 gm.	None	+
8	150/95–130/75	120	None	1.018 in 1 test	No cults. made	Subac. glomerulo-neph. 380 gm.	None	+
9	120/50	150	Blood creatinine 3.3 mg. Serum Ca.: 10.2 mg. Serum P.: 8.04 mg.	1.005	No cults. made	Unclassified type nephritis predom.; also mild embol. glomeruloneph. 340 gm.	None	+
10	200/60	64, 60, 75, 69, 81, 129, 135	Blood creatinine; 3.0, 3.3 mg. Serum P.: 9.06, 8.04, 10.4 mg.	1.006–1.010 in 3 tests	6 blood cults.: no growth	Chr. glomerulo-neph. 240 gm.	None	–. Arteriolar nephroscler.
11	190/110–160/90	Blood urea N.: 55, 90	Blood creatinine: 4.6 mg. Serum Ca.: 7.8, 8.4 mg. Serum P.: 7.8 mg. P.S.P. excretion: 10% in 2 hrs.	Concentr. test: 1.010–1.012	3 blood cults.: no growth	Chr. glomerulo-neph. 380 gm.	Penicil.	Not explicitly diagnosed. Chr. glomerulo-neph.
12	140/42	53, 66, 87, 112, 135, 150, 150, 129, 90, 121	Blood urea N.: 75 mg. Blood creatinine: 2.7, 2.4 mg. P.S.P. excretion: less than 20% in 2 hrs.	1.008–1.013 in 5 tests	3 blood cults.: no growth	Chr. and mild embolic glomerulo-neph., principally healed. 400 gm.	Penicil.	+
13	130/80	25, 45, 58, 75	Blood creatinine: 1.8 mg. P.S.P. excretion: 37% in 2 hrs. CO_2 combining power: 24 vol.%	Concentr. test: 1.011–1.018	2 blood cults.: no growth. P.M.: Strep viridans	Embol. glomerulo-neph.; 55% of glom. involved, recent and healing. 380 gm.	Penicil.	+
14	160/80	45, 100, 150, 180	Blood creatinine: 3.8 mg. CO_2 combining power: 24 vol. % P.S.P. excretion test: 5% in 1 hr.	Concentr. test: 1.011	11 blood cults: no growth	Embol. glomerulo-neph., 86% of glom. involved, predom. healing. 400 gm.	Penicil.	+. Ac. fibrin. pericard.

TABLE 1.—CASES OF SUBACUTE BACTERIAL ENDOCARDITIS WITH UREMIA

No.	Blood pressure	Blood concentration non-protein nitrogen mg./100 cc.	Other laboratory data on renal function	Specific gravity of urine	Organism	Renal lesion and kidney weight	Antibiotic therapy	Clinical diagnosis of uremia
				Nephritis Probable or Possible Uremia				
15	180/90	60	None	1.012 (1 test)	No blood cult. P.M.: hemolytic Strep.	Embol. glomeruloneph.; 72% of glom. involved, chiefly healing. 420 gm.	None	—
16	90/80	—	None	1.008 (1 test)	No blood cult.	Chr. glomeruloneph.; a few embol. lesions. 590 gm.	None	—
17	190/120–148/100	31, 37, 40, 36, 53, 60	P.S.P. excretion 11.4% in 2 hrs.	1.020	2 blood cults.: no growth. P.M.: no growth	Ac. glomeruloneph. 420 gm.	None	— Ac. glomeruloneph.
18	90/84	—	None	—	1 blood cult. Neisseria gonorrhoeæ	Ac. glomeruloneph. 500 gm.	None	—
19	110/64	—	None	1.018 (1 test)	No blood cult.	Ac. glomeruloneph. 470 gm.	None	—
20	156/80	—	None	1.011	No blood cult.	Chr. glomeruloneph.; a few embol. lesions. 250 gm.	None	—
21	200/100–156/82	30, 40, 55 60	Blood creatinine; 2.7 mg./100 cc.	Concentr. test: 1.015	3 of 8 blood cults. gram positive cocci	Subac. glomeruloneph. 600 gm.	None	Suggested by one observer Chr. glomeruloneph.
22	94/84	50, 69, 86, 112	None	1.010–1.020	Blood cults. Strep. viridans	Embol. and chr. glomerulonoeph. 600 gm.	None (urea given with sulfadiazine)	Suggested by one observer
23	110/34	Blood urea N.: 50, 58	Serum P. 4.36 mg./100 cc.	1.014–1.022 (in 3 tests)	No blood cult. P.M. Friedlander-like bacillus	Ac. (intercapillary) glomerulonoeph. 500 gm.	Penicil. Streptomycin	—
				Nephritis, Renal Insufficiency without Uremia				
24	200/80	32, 32, 31	P.S.P. excretion 24% in 2 hrs.	Concentr. test: 1.017	1 blood cult.: no growth. P.M.: Strep. viridans	Ac. (intercapillary) glomerulonoeph. 720 gm.	None	— Chr. glomeruloneph.

in 44, and infarcts in 38. In only 36 instances were there no renal lesions other than infarcts in some. The clinical and pathological data concerning renal insufficiency have been summarized in Table 1. In each instance some degree of hematuria, proteinuria or both were present, and for this reason no notation of these is made in the table. A distinction has been made between those cases in which there was sufficient information to establish the diagnosis of uremia, and in those in which it was not possible. The combined weight of the kidneys is given with the *renal lesions*. In the column labelled *Clinical Diagnosis of Uremia*, the diagnosis was explicitly made by the clinical staff. In some instances, such a diagnosis was *implied*; it has, for example, been the clinical practice to diagnose "diffuse" together with embolic glomerulonephritis in cases of subacute bacterial endocarditis that have manifestations of uremia. In several cases, the diagnosis of glomerulonephritis has been made without explicit diagnosis of uremia. In these cases, both facts are stated in this column. In addition, other manifesta-

tions of uremia, such as acute fibrinous pericarditis and coma with urea frost, are recorded.

There were 14 cases of well-established uremia. The lowest blood concentration of non-protein nitrogen in any of these at the time of death was 75 mg. per 100 cc.; the highest was 224. Most commonly it was about 150. In 3 instances, a concentration test had been made, and the ability of the kidney to concentrate the urine was found to be severely impaired. In all the rest but one (acute glomerulonephritis), the casual urine specimens had repeatedly a specific gravity close to 1.012. Phenolsulphonephthalein excretion tests were performed in 5 cases, and in each the clearance was markedly reduced. The concentration of phosphorus in the serum was elevated in the 4 cases in which it was deter-

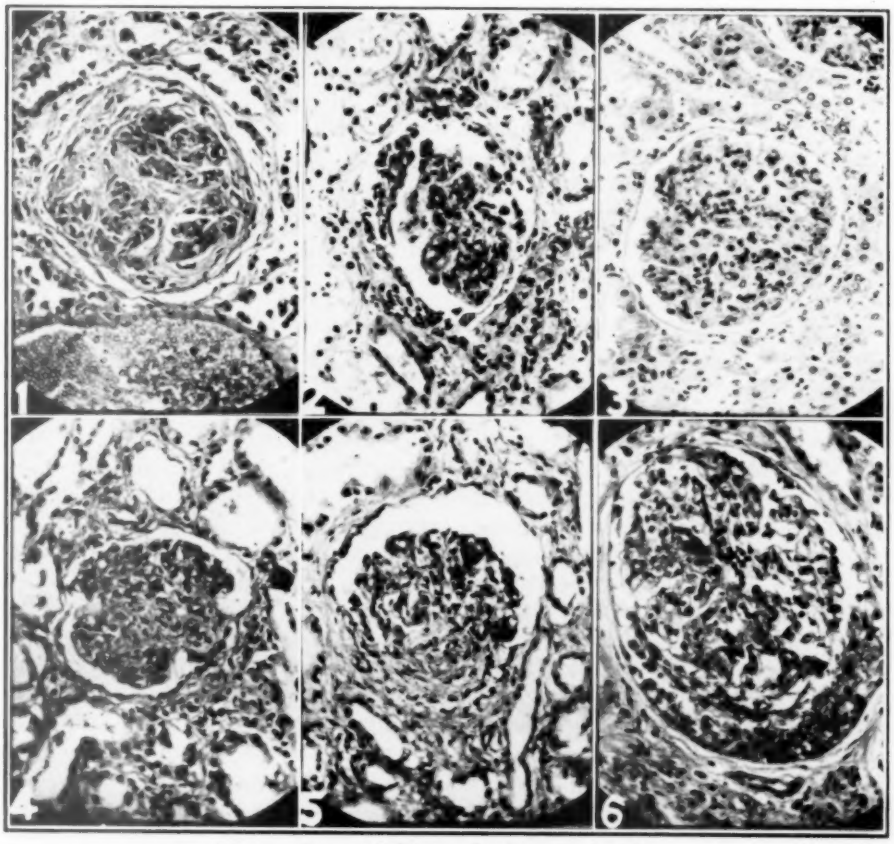


FIG. 1.—Types of Glomerular Lesions (x 200).

1. CASE 10. Chronic glomerulonephritis. Bowman's space obliterated and advanced fibrosis of the tuft.
2. CASE 8. Subacute glomerulonephritis. Well-developed epithelial crescents.
3. CASE 23. Acute glomerulonephritis. The glomerulus fills Bowman's space, is bloodless and has increased cellularity.
4. CASE 22. Chronic glomerulonephritis. Diffuse increase of the fibrous tissue of the tuft.
5. CASE 22. Embolic glomerulonephritis. A fairly characteristic example of a healed focal lesion in the same case as the preceding.
6. CASE 13. Embolic glomerulonephritis. A fresh lesion with hemorrhage into Bowman's space.

mined. Uremia was diagnosed explicitly in 11 of these 14; the other 3 were considered clinically to have chronic glomerulonephritis or arteriolar nephrosclerosis.

In 8 of these 14 cases the renal lesion was that of embolic glomerulonephritis. The lesion was severe in each of these. Recent or healed lesions might predominate in an individual case. In 2 others, embolic lesions were present, but of themselves were not sufficient to account for the renal insufficiency. One of these was accompanied by an unclassified type of nephritis, characterized by a fairly widespread interstitial chronic inflammatory reaction and fibrosis. The other coexisted with chronic glomerulonephritis. There were, in addition, 1 case of subacute and 3 of chronic glomerulonephritis.

The likelihood of uremia was suggested in another 9 cases in which there were insufficient or equivocal clinical data. Cases of severe embolic glomerulonephritis have been excluded from this category of possible uremia because no clinical tests were done. Four of the 9 cases were acute (intercapillary), 1 subacute, and 1 chronic glomerulonephritis. In 3 others there was embolic glomerulonephritis in association with chronic glomerulonephritis; and in another, the only lesion was severe embolic glomerulonephritis.

One case of well-developed intercapillary glomerulonephritis had renal insufficiency as determined by phenolsulphonephthalein excretion and urinary concentration, yet there was no elevation of the non-protein nitrogen concentration of the blood (Case 24).

Blood cultures had been made in 6 of the 9 cases with probable or definite uremia due only to embolic glomerulonephritis and yielded growth of *Strep. viridans* in only 2, although this organism was recovered at necropsy in 1 instance in which the blood was sterile. In another the *post mortem* culture also was successful and a third revealed a hemolytic streptococcus. If all cases of uremia or probable uremia are excluded from the whole series, blood cultures were made in 47 patients, and the *Strep. viridans* was recovered in 30 of these; *N. flava* in 1 case and non-hemolytic streptococci from 3 others, giving an incidence of 72%. Because of the paucity of cases, it cannot be stated definitely that the uremia militates against recovery of the organism, but the results suggest this.* Blood cultures were also reported in the 4 instances of glomerulonephritis with uremia and 3 without proven uremia. In only 2 was the culture positive, once, for *N. gonorrheae* and once for an unidentified gram-positive coccus. *Post mortem* culture was made in 3 of these 7 cases; from 1 there grew the *Strep. viridans*, from 1 a Friedländer-like organism that did not type, and the 3rd was sterile.

Sustained hypertension was observed in 5 of the 11 cases of glomerulonephritis and only 1 with embolic glomerulonephritis. Of the 100 cases 22% had received a variable amount of penicillin, while 78% had not. The incidence of glomerulonephritis and renal insufficiency was 27% in the former and 24% in the latter group.

Discussion. These findings clearly demonstrate that uremia is by no means an infrequent complication of subacute bacterial endocarditis. It occurs as the consequence of embolic, acute, subacute or chronic glomerulonephritis. With the embolic glomerulonephritis the development of uremia requires a fairly severe lesion. The converse is not true; that is, embolic glomerulonephritis in several cases was as widespread and severe as in the group with uremia, but repeated de-

* In preliminary experiments with Dr. William N. Hubbard, Jr. and Miss B. Toharsky, it has not been possible to demonstrate *in vitro* that uremic blood exerts an inhibitory effect on the growth of *Strep. viridans*.

terminations of the blood concentration of non-protein nitrogen failed to reveal azotemia.

The low incidence of positive blood cultures in the cases of glomerulonephritis is in accord with previous observations[4,13] but the conclusion cannot necessarily be drawn that a bacteria-free stage associated with hyperergy to the alpha hemolytic streptococcus is involved in its pathogenesis. The reasons for this are:

1) Blood cultures are less frequently made in cases of uremia, since the likelihood of there being an underlying bacterial endocarditis is not so frequently entertained.

2) In 1 of the 3 cases of glomerulonephritis with negative blood culture during life, the *Strep. viridans* was recovered by culture at necropsy.

3) Uremia itself may make it difficult to recover the organism from the blood.

Although treatment of subacute bacterial endocarditis with penicillin causes the Addis count of the urinary sediment to be reduced, it did not reduce the incidence of all types of nephritis or renal insufficiency in those cases that were not cured. The cases in the present series are too few to evaluate the relative degree of healing of the focal lesions as a consequence of such therapy.

It would appear that subacute bacterial endocarditis due to *Strep. viridans* stands second to beta-hemolytic streptococcus infection as a cause of glomerulonephritis. These considerations should encourage search for, and probably treatment of, subacute bacterial endocarditis in patients who have disease of the valves of the heart, associated with nephritis and fever.

Summary. The clinical records, necropsy protocols and histologic sections of 100 individuals dying of subacute bacterial endocarditis have been reviewed. Definite evidence of severe renal insufficiency with uremia was obtained in 14 cases, and the probability or possibility of its existence in another 9. In 9 of these 23 the lesion in the kidney was embolic glomerulonephritis. In 9 and 1 additional case without uremia, the lesions were acute, subacute or chronic glomerulonephritis. The remaining 5 had a combined type of nephritis. In view of these findings, the diagnosis of subacute bacterial endocarditis should be suggested when evidences of renal insufficiency coexist with valvular heart disease and fever.

REFERENCES

1. **Addis, T.,** and **Oliver, J.:** The Renal Lesion in Bright's Disease, p. 154, New York, P. B. Hoeber, 1931.
2. **Baehr, G.:** J. Exp. Med., **15,** 330, 1912.
3. Idem: Arch. Int. Med., **27,** 262, 1921.
4. **Baehr, G.,** and **Lande, H.:** J. Am. Med. Assn., **75,** 789, 1920.
5. **Bell, E. T.:** Am. J. Path., **8,** 639, 1932.
6. **Bloomfield, A. L., Armstrong, E. D.,** and **Kirby, N. M. M.:** J. Clin. Invest., **24,** 251, 1945.
7. **Boyarsky, S., Burnett, J. M.,** and **Barker, W. H.:** Bull. Johns Hop. Hosp., **84,** 207, 1949.
8. **Boyd, W.:** A Textbook of Pathology, 3d ed., p. 404, Philadelphia, Lea & Febiger, 1938.
9. **Christian, H. A.:** J. Mt. Sinai Hosp., **8,** 427, 1941-42.
10. **Géraud, J.:** Prog. Méd., **11,** 301, 1947.
11. **Goldring, W.,** and **Chasis, H.:** Hypertension and Hypertensive Disease, p. 79, New York, Commonwealth Fund, 1944.
12. **Libman, E.:** Personal communication. Cited by A. M. Fishberg in Hypertension and Nephritis, 4th ed., p. 551, Philadelphia, Lea & Febiger, 1939.
13. **Libman, E.,** and **Friedberg, C. K.:** Subacute Bacterial Endocarditis, p. 42, New York, Oxford Univ. Press, 1941.
14. **Löhlein, M.:** Med. Klin., **6,** 375, 1910.
15. **Pedro-Pons, A.,** and **Ferrer, N. M.:** Med. Clin., Barcelona, **8,** 12, 1947.
16. **Russell, D. S.:** A Classification of Bright's Disease, p. 76, Med. Res. Council, London, 1929.

STUDIES OF IMMUNOLOGICAL REACTIONS OF PATIENTS WITH HODGKIN'S DISEASE: THE TUBERCULIN REACTION

By Antonio Rottino, M.D.

AND

George T. Hoffmann, M.D.

NEW YORK, NEW YORK

(From the Hodgkin's Disease Research Laboratory*, St. Vincent's Hospital, New York)

Prior to 1924 many workers had attempted to establish a possible relationship between Hodgkin's disease and tuberculosis on the basis of the tuberculin skin test. Though these attempts were unsuccessful, they brought to light an interesting fact, namely, that patients with Hodgkin's disease were as a rule anergic to tuberculin.

As early as 1904 Reed[6] noted that reaction of Hodgkin's disease patients to tuberculin was negative in 5 of 8 instances. In 1928 Bastai[1] reported 22 negative reactors in a group of 24 Hodgkin's disease patients receiving tuberculin solution (their titers being as low as 1:30) and a weak positive reaction in 2 cases. He further observed that a negative reaction had no relationship to the previous duration of the disease or to its stage of advancement, and also that an occasional negative reactor became positive after a remission had been induced by Roentgen-ray therapy.

In 1930 Marquio[4] observed that patients suffering from concurrent Hodgkin's disease and tuberculosis reacted negatively to tuberculin.

A further contribution was made by Steiner[7] in 1934 when he modified the usual technique by employing tuberculin protein instead of old tuberculin and by using 3 different titers of this on each patient—1:10,000; 1:1,000; and 1:100. The results he observed were in essential agreement with those of earlier workers. Furthermore, from repeated tests on the same patient, he found that negative reactors remained negative after Roentgen-ray therapy, that in those instances where the reaction was positive it was weak, and that the next-larger dose induced no greater reaction than the smaller dose.

In 1940 de Marval[5] recorded the development of anergy in tuberculosis patients with concurrent Hodgkin's disease. He attempted to immunize Hodgkin's patients with BCG and found that this failed to produce a positive tuberculin reaction. He found also that as much as 2 cc. of crude tuberculin could be injected into

TABLE I.—THE TUBERCULIN REACTION IN HODGKIN'S DISEASE COMPARED WITH A CONTROL GROUP OF NORMAL PERSONS

	Number of Cases	*Positive 1:10,000*	*Positive 1:100*	*Total Positive*
Hodgkin's Disease	57	3 (5%)	8 (14%)	19%
Control	248	42 (17%)	49 (20%)	37%

* Supported in part by grants from the National Cancer Institute of the U. S. Public Health Service, the American Cancer Society, and the Damon Runyon Memorial Fund.

Hodgkin's disease patients with no reaction becoming apparent.

In 1947 Dubin[3] confirmed this phenomenon of tuberculin anergy of Hodgkin's disease sufferers. He also pointed out that these same patients were poor reactors to the Wassermann test, and showed that their production of protective antibodies against typhoid and brucella infection was below normal.

In 1949 Bostick[2] reviewed 150 cases of Hodgkin's disease from the files of the University of California Hospital. The tuberculin test had been done on 34 patients, 31 of whom had reacted negatively and 3 positively.

Report of our own work follows:

Method of Study. Over a period of 2 years we did tuberculin tests on 57 patients with established Hodgkin's disease, these patients being attendants at our Hodgkin's disease clinic at St. Vincent's hospital. Old tuberculin supplied by the New York City Department of Health was used. It was intradermally injected at 3-day intervals in graduated doses of 0.01 mg., 0.1 mg., and 1.0 mg. As controls, 248 student nurses were tested in an identical manner; the nurses were chosen as controls because as a group the average age approximated that of the patient group.

Results: The results are summarized in the table. Eleven (19%) of the patients reacted positively, as compared with 91 (37%) of the control group. Of the 11 patients, 3 reacted strongly to 1:10,000 dilution; 1 reacted to a 1:1,000 dilution; the others reacted positively only when the concentration was 1:100.

Four of the 11 patients who during initial testing reacted positively, later became anergic to the tuberculin in 2 months, 3 months, 5 months and 8 months respectively. One of these patients had reacted strongly to a dilution of 1:10,000.

Fifteen of the 57 patients died and were autopsied, including 4 of those who had reacted positively to tuberculin. Of these 4, none showed tuberculosis. One patient who had reacted negatively to tuberculin 2 weeks before death was shown at autopsy to have had miliary tuberculosis and tuberculous adenitis in addition to Hodgkin's disease.

Discussion. Our findings agree with those of earlier workers, all of whom pointed out that the majority of persons with Hodgkin's disease do not react to tuberculin. None of our patients who reacted negatively ever regained their allergy even during remissions induced by Roentgen-ray or nitrogen mustard therapy. In fact, the positive reactors who later became negative did so despite administration of these therapies. Our attempt to correlate tuberculin sensitivity or lack of sensitivity to duration of disease, tempo of disease, or histologic pattern were fruitless. The attempts at histologic correlation were not, however, completely satisfying as in many instances it had not been possible to perform the biopsy and administer the tuberculin simultaneously.

One observation which surprised us was the relative rapidity with which allergy might be lost. In 1 instance this occurred after only 2 months. In 2 instances where repeated tests were done at short intervals there was first a lessening of the intensity of the reaction before it disappeared completely, indicating a progressive loss of allergy rather than an abrupt cessation. Performance of multiple tests made it possible to observe a shift in the percentage of positive reactors from an initial 19% to 12%. This change depended upon rearranging the statistics to include as negative reactors 4 patients who had at first been positive.

The relatively low incidence of the tuberculin reaction in our control group may be subject to question. However, our results are in harmony with those obtained among students at the University of Chicago (see U. S.

Public Health Report, January, 1949), where the positive reaction was 27% as compared with 37% in our group.

The significance of the altered allergic state during Hodgkin's disease is not understood. There must be some relation between the diseased state of the lymphoreticuloendothelial system and this alteration of allergy, but what manner of relationship and which specific element is involved is not clear.

Conclusions. 1. A study of the reaction of patients with Hodgkin's disease to tuberculin indicates the development of an anergic state.

2. The cause of this is not known.

3. Because some persons with Hodgkin's disease react strongly to tuberculin and a large percentage of normal persons react negatively, the test is not of itself sufficiently indicative to be of great diagnostic aid. It is, however, of value when used to supplement other clinical data.

REFERENCES

1. **Bastai, P.:** Klin. Woch., **7**, 1606, 1928.
2. **Bostick, W. L.:** Calif. Med., **70**, 1, 1949.
3. **Dubin, I. N.:** Ann. Int. Med., **27**, 898, 1947.
4. **Marquio, L.:** Arch. argent. de Pediat., **1**, 573, 1930.
5. **de Marval, L.:** Prensa med. argent., **27**, 2310, 1940.
6. **Reed, D. M.:** Johns Hop. Hosp. Rep., **10**, 133, 1902.
7. **Steiner, P. E.:** Arch. Int. Med., **54**, 11, 1934.

INHIBITION OF HYALURONIDASE, USING FLUORESCEIN AS AN INDICATOR

By Charles R. Shuman, M.D.
INSTRUCTOR IN MEDICINE
PHILADELPHIA, PENNSYLVANIA

(From the Department of Medicine, Temple University School of Medicine)

The use of fluorescein as an indicator in the study of hyaluronidase inhibitors *in vivo* occurred to the author in the light of observations by Bukantz and Dammin[2] who used the dye in evaluating the effect of antihistaminic agents upon the action of histamine. In previous studies, the local action of the spreading factors *in vivo* was gauged by other dyes, including India ink, Evans blue, trypan blue[7,14,40], all of which produce areas of persistent discoloration lasting for long intervals, the spreading effect being proportional to the area covered by the dye. The advantage of fluorescein appeared to be that it remained visible for an hour or less when injected alone, but disappeared rapidly when hyaluronidase* was added to it. This difference in duration and intensity of fluorescence with and without the enzyme permitted evaluation of the extent to which various factors were or were not antagonistic to the enzyme used. The tests described here involve the use of a testicular hyaluronidase in studying the action of salicylates and other hyaluronidase antagonists *in vivo*, and in observing the possible usefulness of this method in testing rheumatic fever patients with hyaluronidase of streptococcal origin as antigen, to see if such patients had a circulating antibody to the specific enzyme. Use is also being made of this technique in the study of adrenal cortical stimulation and of steroid hormone inhibitors of hyaluronidase.

Inhibition of the spreading effect of hyaluronidase in animals and humans receiving salicylates was originally shown by Guerra[14]; however, Pike[34] was unable to demonstrate any effect of sodium salicylate upon hyaluronidase activity *in vitro*. The work of Dorfman *et al.*[6] confirmed the observations that relatively low blood levels of salicylates in rabbits inhibited the spread of India ink injected with hyaluronidase into the skin. At the same time, it was shown that *in vitro* inactivation of the enzyme did not occur until very high concentrations of salicylate were obtained; at the usual therapeutic range of salicylate concentration there was no effect upon hyaluronidase activity. It is probable that this discrepancy between *in vivo* and *in vitro* results is due to the formation of active conjugated forms of salicylate produced within the body and recoverable from the urine as described by Meyer[30].

The study of the spreading factors began in 1929 with the discovery by Duran-Reynals[8] that a testicular extract produced rapid local dissemination of vaccinia virus and other substances. He subsequently showed that the spreading factor was antigenic. McClean[24] independently found that testicular extract was effective in altering tissue permeability. Chain and

* Wydase, Supplied by Wyeth Inc., Philadelphia.

Duthie[4] established the common identity of the spreading factor and the mucolytic enzyme which Meyer[29] found to be active in causing hydrolysis of hyaluronic acid, a mucopolysaccharide isolated originally from the vitreous humor[27] and subsequently shown to be related to the mucopolysaccharides forming the ground substance of connective tissue. An extensive description of the properties and occurrence of hyaluronic acid in nature has been made by Meyer[26].

Studies of the antigenicity of the spreading factor by McClean[23] showed that the antiserum to bull testis was not effective against the enzyme antigen from the mouse testis or from bacteria. The specificity of the antigenic activity of hyaluronidase has been widely studied and a recent discussion of bacterial enzymes in the production of specific antisera has been presented by Thompson and Moses[42]. With respect to infectious diseases caused by hyaluronidase-producing bacteria, there has not been any satisfactory correlation between the virulence of the organism and its ability to produce the enzyme[10,19,43]. Friou and Wenner[11] demonstrated antihyaluronidase activity in the serum of patients with streptococcal infections and noted that antibody titers were higher in the rheumatic fever group than in other post-streptococcal diseases. These observations were confirmed and extended by Quinn[35] and Harris[16]. The purpose of the present study is to describe a method which may be adapted for the *in vivo* determination of these factors.

Method. Fluorescein dye was diluted with saline to a concentration of 1:50000, and a solution of sodium salicylate was prepared in the strength of 40 gamma per 0.1 cc. The enzyme employed in the test was derived from bull testis and was prepared by dissolving 150 turbidity-reducing units in 1 cc. of saline. Injections were made with a clean tuberculin syringe, using ¾ inch, 26-gauge needles. The sites injected were located in corresponding areas in patients and controls. The volume of material injected varied from 0.08 cc. to 0.12 cc. in different experiments, in order to avoid excessive local pressure in the spreading effect.

Intracutaneous wheals were made on the volar surface of the forearm in most instances, first using fluorescein alone, in order to estimate the disappearance time without the enzyme; then repeating the test with the enzyme added. Several tests were done using the salicylate solution to examine the effect of the drug injected locally upon hyaluronidase activity. Patients tested included those with rheumatic fever, rheumatoid arthritis, peripheral vascular disease, and patients receiving salicylate therapy. In the latter group, blood salicylate levels were determined at the time of the test. Control tests were done simultaneously on normal individuals. The fluorescence was originally evaluated under filtered ultraviolet light, but subsequent observations were made in reflected daylight, the yellow color of the dye becoming apparent when the skin was gently stroked with an alcohol-saturated cotton sponge. The end-point was accepted as that time when no color could be appreciated in the injected areas. The spread of the dye was not evaluated, because it rapidly disappeared from the skin when the enzyme was present. It is possible that the use of the dermofluorometer would aid in accurate measurement of hyaluronidase antagonists *in vivo* as suggested in the histamine study[2].

Results. Patients receiving salicylates showed a delay in the disappearance time of the fluorescein indicator when hyaluronidase was injected with it, in contrast to the rapid disappearance noted in controls and in patients not receiving salicylates. Those patients having the highest blood levels at the time of testing produced the greatest retardation of the disappearance time and required a higher concentration of the enzyme in order to reduce the time to that of the controls. Patients with rheumatic fever, rheumatoid arthritis, and other diseases, who were not receiving medications when tested, showed no inhibition of enzyme activity; in these patients as in the control group the average times for dye disappearance were 4½ minutes and 4

minutes respectively. On 5 occasions it was possible to test patients prior to, or several days following, a course of salicylates, as well as during the treatment period. The disappearance time of the dye using 0.01 cc. of enzyme increased from 4½ minutes to an average of 24 minutes while the drug was being administered, indicating that inhibition of the spreading effect had occurred, permitting the dye to remain visible in the skin for a period of time approaching that of the dye injected alone (Tables 1, 2, and 3).

The effect of salicylate injected locally with hyaluronidase in the presence of fluorescein was to increase slightly the rate of dye disappearance rather than to delay it, as occurred with orally administered salicylate. Small amounts of salicylate were employed in various concentrations in an effort to inactivate the enzyme at the site of injection; that this would not occur was anticipated from the work of Meyer[30]. In higher concentrations, salicylate produced a hyperemia at the site of injection and increased the rate of disappearance from 40 minutes for fluorescein alone to 14 minutes when salicylate and fluorescein were injected together (Table 1). This effect is apparently produced by the local irritating effect of the salicylate.

The antihistaminic drugs, thephorin and pyribenzamine, were administered orally to several patients (Table 5) to evaluate their effect upon hyaluronidase injected intracutaneously. The use of these drugs therapeutically in cases of urticaria and allergic skin manifestations where histamine may be an important factor suggested that if histamine were liberated at the site of injection of hyaluronidase it could be neutralized and a delay in the spreading action produced with these drugs. The failure of any delay in the disappearance time of the dye to occur in these patients receiving antihistaminic drugs is considered as evidence against histamine playing a part in the hyaluronidase action.

The enzyme was then used in a patient with extensive thromboangiitis obliterans and was injected into a pregangrenous area at the base of the toes and several other locations including the left hand where a positive Allen's sign was present. The dye was found to disappear at the same time from all the injected areas, including the ischemic site, as well as from the hand either when the radial artery was patent or when obliterated by pressure. Capillary fragility tests were performed by Gothlin and Rumple-Leede methods following an intracutaneous injection of hyaluronidase into several normal persons. The number of petechiae counted in the injected areas did not differ significantly from the number counted in adjacent areas. These observations were considered as evidence opposed to the proposal that hyaluronidase has a direct effect upon vascular structures which will increase capillary fragility[3].

One case of porphyria receiving no treatment showed a very rapid disappearance time both with the enzyme and without it. The dye disappeared in 17 minutes (average normal, 40 minutes) and with the enzyme added to it the time decreased to 3 minutes (average normal, 4 minutes) (Table 1). It was noted that any injection given to the patient was surrounded by an area of intense hyperemia which suggested that local factors of blood flow and tissue irritability were of importance in evaluating the results of the procedure. There was no measurable inhibition of hyaluronidase *in vivo* by the porphyrins which were present in high concentration in the urine.

A method for estimating roughly the amount of inhibition of the enzyme was accomplished by increasing the concentration of hyaluronidase in the

dye solution (Table 3). The amount of hyaluronidase inhibition present in salicylate-treated patients was assayed by increasing the enzyme content of the skin test until the disappearance time of the dye was equal to that of a control patient injected with 0.01 cc. of enzyme. In one case (M.K.), receiving both salicylate and quinidine, it was necessary to increase the hyaluronidase concentration 14 times that of the control to produce an equivalent disappearance time. In another instance (J.M.), while on salicylates, 6 times as much hyaluronidase was required to produce a disappearance time of 5 minutes as when the same patient was not taking the drug. Several such observations were made, but there are insufficient data at this time to attempt a correlation between the blood salicylate levels and the amount of hyaluronidase inhibition found in these patients.

Discussion. A recent report by Fulton[12] confirms previous evidence of Dorfman[6], and of Pike[34] to the effect that salicylate does not inhibit the hydrolysis of hyaluronic acid by hyaluronidase *in vitro*. However, the

TABLE 1.—DISAPPEARANCE TIME OF FLUORESCEIN, AND OF FLUORESCEIN, HYALURONIDASE, AND SODIUM SALICYLATE IN COMBINATIONS, INJECTED INTRACUTANEOUSLY, IN 4 NORMALS, 24 NON-RHEUMATIC CONTROL PATIENTS, AND 1 PATIENT WITH ACUTE PORPHYRIA.

				Disappearance Time in Minutes in 29 Subjects						
	Agent and Dose (in cc.)			*Normals*				*24 non-rheumatic*		*Acute*
Tests	*Fluor.*[1]	*Hyal.*[2]	*Sod. Sal.*[3]	*A*	*B*	*C*	*D*	*Mean*	*Range*	*Porphyria*
(1)	0.1 cc.	—	—	39	36	42	34	40	36 to 62	17
(2)	0.09	0.01	—	5	4	4	4	4	3½ to 5	3
(3)	0.08	0.01	0.02	3	4	3	3		—	—
(4)	0.09	—	0.02	14	13	14	13½		—	—
(5)	0.06	—	0.06	10	8	11	12		—	—
(6)	0.06	0.01	0.06	3	3	2	2		—	—

1. Fluor.—Fluorescein 1:50,000 solution in saline
2. Hyal.—Testicular hyaluronidase (Wydase) 150 Turbidity reducing units per cc.
3. Sod. Sal.—Sodium Salicylate solution: 400 gamma per 0.1 cc.

TABLE 2.—DISAPPEARANCE TIME OF FLUORESCEIN AND HYALURONIDASE INJECTED INTRACUTANEOUSLY IN UNTREATED RHEUMATIC FEVER AND IN SALICYLATE-TREATED PATIENTS.

			Disappearance Time in Minutes					
	Agent & Dose (cc.)		*Untreated Rheumatic Fever (8 patients)*		*Salicylate-treated patients** *L.E.*[3]	*Rh.F.*[4]	*Rh.Ar.*[5]	
Tests	*Fluor.*[1]	*Hyal.*[2]	*Mean*	*Range*	*Mean*	*Range*		
(1)	0.10	—	48	34 to 72	52	32 to 63		
(2)	0.09	0.01	4½	3½ to 6	24	14 to 58		

1. Fluor.—Fluorescein 1:50,000 solution in Saline
2. Hyal.—Testicular hyaluronidase (Wydase) 150 TRU/cc.
3. L.E.—Lupus erythematosus—2 patients
4. Rh.F.—Rheumatic fever—16 patients
5. Rh.Ar.—Rheumatoid arthritis—4 patients

* Blood salicylate levels—10.5 to 21.9 mg./100 cc.

TABLE 3.—DISAPPEARANCE TIME OF FLUORESCEIN AS INFLUENCED BY INCREASING CONCENTRATIONS OF HYALURONIDASE IN SALICYLATE-TREATED PATIENTS.

Agent & Dose (cc.)		*Disappearance Time in Minutes*								
Fluor.[1]	*Hyal.*[2]	*24 Non-treated patients*	*L.E.*[3]	*Rh.H.D.*[4]	*Rh.A.*[5]	*R.F.*[6]	*R.F.*[7] *a*	*R.F.*[7] *b*	*R.F.*[8] *a*	*R.F.*[8] *b*
0.1	0	40	61	34	42	37	41	39	42	44
0.09	0.01	4	36	22	16	14	5	20	24	4
0.08	0.02	4	31	—	—	8	4	11	—	—
0.06	0.04	—	28	16	12	5	—	8	—	—
0.04	0.06	—	21	12	9	—	—	4	—	—
diluent	0.1	—	4	10	4	—	—	—	—	—
diluent	0.14	—	—	3½	—	—	—	—	—	—

1. Fluor.—Fluorescein 1:50,000 solution in saline
2. Hyal.—Testicular hyaluronidase (Wydase) 150 TRU/cc.
3. L.E.—Lupus erythematosus, salicylate level, 14.6 mg./100 cc.
4. Rh.H.D.—Rheumatic heart disease, salicylate level, 19.5 mg./100 cc. receiving quinidine sulphate 0.4 gm. q.i.d.
5. Rh.A.—Rheumatoid arthritis, salicylate level, 18.5 mg./100 cc.
6. R.F.—Rheumatic fever, recurrent, salicylate level, 12.2 mg./100 cc.
7. R.F.—Rheumatic fever. (*a*) Pre-salicylate. (*b*) Salicylate level—10.5 mg./100 cc.
8. R.F.—Rheumatic fever. (*a*) Salicylate level—21.9 mg./100 cc. (*b*) 4 days after salicylate discontinued.

TABLE 4.—EFFECT OF ORAL ANTIHISTAMINICS ON DISAPPEARANCE TIME OF FLUORESCEIN, SHOWING ABSENCE OF HYALURONIDASE INHIBITION.

Agent & Dose (cc.)		*Disappearance Time in Minutes in 3 patients before and during anti-allergic therapy*					
		Thephorin 50 mg. q.i.d.		*Pyribenzamine 100 mg. q.i.d.*		*Thephorin 50 mg. q.4.h.*	
Fluor.[1]	*Hyal.*[2]	*Before*	*During*	*Before*	*During*	*Before*	*During*
0.1	0	36	32	52	60	42	40
0.08	0.02	5	5½	4½	4	3½	4

1. Fluor.—Fluorescein 1:50,000 solution in saline
2. Hyal.—Testicular hyaluronidase (Wydase) 150 Turbidity reducing units per cc.

TABLE 5.—EFFECT OF INTRAVENOUS HEPARIN ON THE DISAPPEARANCE TIME OF FLUORESCEIN AND HYALURONIDASE, SHOWING PARTIAL INHIBITION OF HYALURONIDASE ACTIVITY.

Agent & Dose (cc.)		*Disappearance Time in Minutes in 2 patients receiving heparin*			
		Heparin 500 mg. in 500 cc. continuous I.V.		*Heparin 50 mg. I.V. q.4.h.*	
Fluor.[1]	*Hyal.*[2]	*Before*	*During*	*Before*	*During*
0.1	—	38	38	53	—
0.09	0.01	4	16	4½	22

1. Fluor.—Fluorescein 1:50,000 solution in saline
2. Hyal.—Testicular hyaluronidase (Wydase) 150 Turbidity reducing units per cc.

observations of Guerra[14] indicating that antagonism to hyaluronidase action was effected by salicylates in rabbits and humans were substantiated by Dorfman[6]. Sodium salicylate given in dosage sufficient to produce therapeutic blood levels resulted in the inhibition of the spreading activity of hyaluronidase in rabbits. Graded increments of salicylates *in vitro* did not inhibit the enzyme until levels were reached which were capable of denaturation of protein. The lack of effect of salicylate injected locally with the enzyme has been observed previously and suggested that certain conjugation products of salicylate metabolism may be responsible for the inhibition observed. Gentisic acid and related compounds capable of inhibiting the enzyme were recovered from the urine of patients receiving salicylates orally[30]. It is not clear as to what effect is produced by salicylates *in vivo* upon the hyaluronic acid-hyaluronidase system that is not demonstrable *in vitro*. That salicylate has an inhibitory effect upon antibody formation has been clearly shown by Swift[39], Jager[20], and Homberger[18] using different types of antigenic stimuli. However, the inhibition of testicular hyaluronidase by salicylate-treated patients is not due to the presence of antibody. Salicylates may inhibit the spreading effect of the enzyme *in vivo* by activation of the pituitary-adrenal system which has been shown to inhibit hyaluronidase[33,37]. However, a direct action of a metabolically active product of salicylate upon the collagen altering its reaction to hyaluronidase should be studied.

The influence of hyaluronidase upon capillary permeability has been subject to investigation by Duran-Reynals[7], Meyer[26], Chambers[3], and others[1,9]. Chambers was unable to detect any changes in the rate of edema formation with testicular extract (Chambers quoted in Duran-Reynals[7]; no change in blood pressure has been found with its use intra-arterially in animals[5]. However, Duran-Reynals[7] and later Aylward[1], found that the rate of passage of dye into the tissues when injected with hyaluronidase was much increased. Recently Elster, Freeman, and Dorfman[9], using T 1824 with testicular extract administered intravenously, noted the occurrence of edema and the rapid diffusion of dye into certain tissues of rats, indicating an increase in capillary permeability. On the other hand, Meyer and Ragan[31] state that increased capillary permeability was not present when the

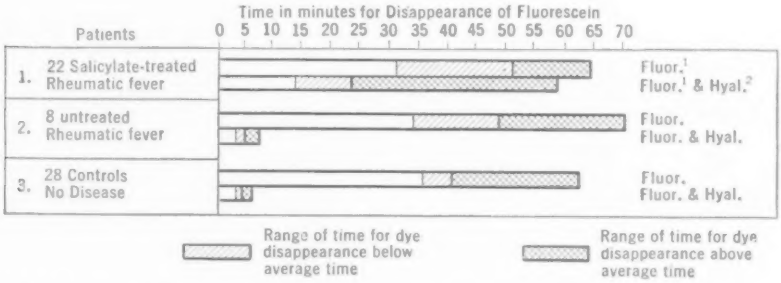

Time in minutes for Disappearance of Fluorescein
Patients
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70
1. 22 Salicylate-treated Rheumatic fever
Fluor.[1]
Fluor.[1] & Hyal.[2]
2. 8 untreated Rheumatic fever
Fluor.
Fluor. & Hyal.
3. 28 Controls No Disease
Fluor.
Fluor. & Hyal.
Range of time for dye disappearance below average time
Range of time for dye disappearance above average time

FIG. 1.—Inhibition of Hyaluronidase in 22 Salicylate-treated Rheumatic fever patients, as compared to 8 untreated cases and 28 controls.

1. Fluor.—Fluorescein 1:50,000 solution in saline.
2. Hyal.—Testicular hyaluronidase (Wydase) 150 Turbidity reducing units per cc.

enzyme was injected intravenously, but could be produced by injecting it into the tissues. In the present work it was not possible to demonstrate any increase in capillary fragility using the positive pressure methods following the injection of varying concentrations of the enzyme intracutaneously and counting the number of petechiae in the area of injection 15 minutes later. In addition, a patient with extensive thromboangiitis obliterans showed no difference in reaction to the enzyme in areas of severe involvement (pregangrenous) as compared with normal areas of skin. Thus, hyaluronidase in concentration sufficient to produce a rapid disappearance of the indicator dye did not have any appreciable effect upon capillary permeability in these observations. Swyer[40] was not able to demonstrate any significant change in permeability of capillaries, using testicular hyaluronidase. In the investigations of Wislocki *et al.*[44], although histochemical studies revealed metachromatic staining within the walls of blood vessels indicating the presence of mucopolysaccharides, digestion of these tissues in hyaluronidase failed to destroy the metachromasia, indicating the absence of hyaluronic acid or substances susceptible to the enzyme in these tissues.

In patients treated with antihistamines, the effect of hyaluronidase injected with fluorescein indicator was not different from that in normal controls. This result is to be contrasted to those found in patients tested with histamine intracutaneously, using the same indicator, wherein its rapid disappearance was reversed by the use of antihistaminic agents[2]. These results would indicate that the modes of action of hyaluronidase and histamine locally are different, since the latter is inactivated by antihistaminic drugs, while the former is not. It was considered as possible that the local injection of the enzyme and indicator may release histamine at the site. This would explain the discrepancy between the *in vivo* and *in vitro* findings concerning the effect of salicylates. However, the lack of any antagonism to hyaluronidase by the antihistaminic drugs tends to exclude this possibility. In contrast, Mayer and Kull[22] found that antihistamines injected into rats did reduce the area of spread of India ink by hyaluronidase; and Swyer[40] stated that the spreading action of hyaluronidase was potentiated by histamine and that this latter agent was inhibited by the administration of salicylate. None of the present findings are in accord with these views.

There have been some observations to indicate that estrogenic and adrenal cortical hormones may be important factors in the regulation of tissue permeability, perhaps through the action of these hormones upon the mucoid ground substance. Estrones have been shown to increase the hyaluronic acid content of the sexual skin of monkeys during the estrous cycle[32]; and Sprunt[38] demonstrated inhibition of the spread of India ink injected with hyaluronidase in rabbits treated with estrogen or in pseudopregnancy. It has been shown that the spreading effect of hyaluronidase is enhanced considerably by removal of the adrenal glands in animals and that this defect is correctable by the administration of adrenal cortical extract[33].

In the human, activation of the adrenal cortex by epinephrine, or by surgery, will inhibit the spreading effect of hyaluronidase as determined by fluorescein disappearance[37]. Preliminary observations with ACTH and cortisone show marked inhibitory effects upon hyaluronidase activity[36]. A more detailed discussion of these findings is in preparation. Employing the viscosimetric method for the detection of inhibition of hyaluronidase,

Hakanson and Glick[15] were unable to correlate levels of inhibition detected in the sera of females with sex hormonal changes occurring during various phases of the menstrual cycle or during the various stages of pregnancy.

In performing this test we have employed only an enzyme of testicular origin and have observed no antagonism to it in patients with post-streptococcal diseases in whom the titers of antihyaluronidase should be elevated. It will be necessary to test for these specific antihyaluronidases by means of enzyme obtained from the streptococcus, pneumococcus, and the other organisms capable of producing them and for which specific antibodies are found in the course of diseases due to these organisms[42]. Good and Glick[13] found serum inhibitor for hyaluronidase to be high in acute rheumatic fever. Unlike antibody which is specific for certain hyaluronidases, serum inhibitor is non-specific in its action. It is more heat labile, has a lower electrophoretic charge density than the antibody[13]; and can be more accurately measured by the viscosimetric method. In untreated rheumatic fever patients, no effect of serum inhibitor could be detected by the fluorescein disappearance method described here.

Salicylate-treated patients have invariably neutralized the spreading factor to some extent when the enzyme is injected intracutaneously with a fluorescein dye indicator. The degree of inactivation was semi-quantitated in several patients, showing that approximately 6 to 14 times as much enzyme was necessary to cause the disappearance of the dye in salicylate-treated patients as in untreated controls.

Observations made upon patients receiving heparin in the treatment of thromboembolic disease indicate that shortly after the injection of heparin intravenously the enzyme is inactivated, so that the dye remains in the skin for a longer interval. As the Lee-White clotting time returns to normal, this effect is lost and the dye is rapidly dissipated by the enzyme. Previous observations have shown that heparin is antagonistic to the action of hyaluronidase *in vitro*[25,26]. Swyer[41] demonstrated that heparin in low concentration was inhibitory to hyaluronidase *in vitro*, while very high concentrations of salicylate were necessary for similar inhibition of the enzyme.

Summary. Employing a fluorescein dye indicator, injected intracutaneously, it was possible to evaluate the spreading action of hyaluronidase injected with the dye.

1. In patients treated with salicylates, the action of hyaluronidase was found to be inhibited. The disappearance time of the dye was markedly delayed in these patients.

2. A dilute solution of sodium salicylate, injected locally with the enzyme and indicator dye, increased the rapidity of dye disappearance because of the local irritative effect.

3. Testicular hyaluronidase was not inhibited in untreated patients with acute rheumatic fever, in whom the titers of streptococcal antihyaluronidase as well as serum inhibitor are presumed to be increased.

4. During the course of administration of antihistaminic drugs, there was no inhibition of hyaluronidase.

5. Intravenous heparin appeared to inhibit the action of hyaluronidase somewhat whereas the venous clotting time was prolonged.

6. No effect of the spreading factor on cutaneous capillary fragility could be demonstrated by the use of fragility tests, nor in a patient with extensive vascular disease.

7. Preliminary observations indicate that this method may be useful in evaluating the effect of various steroid hormones, or adrenal cortex stimulation upon the action of hyaluronidase in the human.

REFERENCES

1. **Aylward, F. W.:** Proc. Soc. Exp. Biol. and Med., **49,** 342, 1942.
2. **Bukantz, S. C.,** and **Dammin, G. J.:** Science, **107,** 224, 1948.
3. **Chambers, R.,** and **Zweifach, B. W.:** Physiol. Rev., **27,** 426, 1947.
4. **Chain, E.,** and **Duthie, E. S.:** Nature, **144,** 977, 1939.
5. **Christensen, J. E.:** J. Path. and Bact., **48,** 287, 1939.
6. **Dorfman, A., Reimers, E. J.,** and **Ott, M. L.:** Proc. Soc. Exp. Biol. and Med., **64,** 357, 1947.
7. **Duran-Reynals, F.:** Bact. Rev., **6,** 197, 1942.
8. Idem: J. Exp. Med., **50,** 327, 1929.
9. **Elster S. K., Freeman, M. E.,** and **Dorfman, A.:** Am. J. Physiol, **157,** 429, 1949.
10. **Evans, D. J.:** J. Path. and Bact., **57,** 75, 1949.
11. **Friou, G. J.,** and **Wenner, H. A.:** J. Inf. Dis., **80,** 185, 1947.
12. **Fulton, J. K., Marcus, S.,** and **Robinson, Wm. D.:** Proc. Soc. Exp. Biol. and Med., **69,** 258, 1948.
13. **Good, R. A.,** and **Glick, D.:** J. Infect. Dis., **86,** 38, 1950.
14. **Guerra, F.:** Science, **103,** 686, 1948.
15. **Hakanson, E. V.,** and **Glick, D.:** J. Clin. Invest., **28,** 713, 1949.
16. **Harris, T. N.,** and **Harris, S.:** Am. J. Med. Sci., **217,** 174, 1949.
17. **Haas, E.:** J. Biol. Chem., **63,** 163, 1946.
18. **Homburger, F.:** Proc. Soc. Exp. Med. and Biol., **61,** 101, 1946.
19. **Humphrey, J. H.:** Biochem. J., **37,** 460, 1943.
20. **Jager, B. V.,** and **Nickerson, M.:** Am. J. Med., **3,** 408, 1947.
21. **Lange, K.,** and **Boyd, L. A.:** Arch. Int. Med., **74,** 175, 1944.
22. **Mayer, R. L.,** and **Kull, F. C.:** Proc. Soc. Exp. Biol. and Med., **66,** 392, 1947.
23. **McClean, D.:** Biochem. J., **37,** 169, 1943.
24. Idem: J. Path. and Bact., **34,** 459, 1930.
25. Idem: Ibid., **54,** 284, 1942.
26. **Meyer, K.:** Physiol. Rev., **27,** 335, 1947.
27. **Meyer, K., Dubois, R.,** and **Smyth, E. M.:** Proc. Soc. Exp. Biol. and Med., **34,** 816, 1936.
28. **Meyer, K., Hobby, G. L., Chaffee, E.,** and **Dawson, M. H.:** J. Exp. Med., **71,** 137, 1940.
29. **Meyer, K., and Palmer, J. W.:** J. Biol. Chem., **107,** 629, 1934.
30. **Meyer, K.,** and **Ragan, C.:** Fed. Proc., **7,** 173, 1948.
31. Idem: Mod. Concepts Cardiovasc. Dis., **2,** 17, 1948.
32. **Ogston, A. G., Philpot, J. St. L.,** and **Zuckerman, S.:** J. Endocrinol., **1,** 231, 1939.
33. **Opsahl, J. C.:** Yale J. Biol. and Med., **21,** 255, 1949.
34. **Pike, R. M.:** Science, **105,** 391, 1947.
35. **Quinn, R. W.:** J. Clin. Invest., **27,** 471, 1948.
36. **Shuman, C. R.:** Personal Observations.
37. **Shuman, C. R.,** and **Finestone, A. J.:** Proc. Soc. Exp. Biol. and Med., **73,** 258, 1950.
38. **Sprunt, D. H.,** and **McDearman, S.:** Endocrinology, **25,** 308, 1939.
39. **Swift, H. F.:** J. Exp. Med., **36,** 735, 1922.
40. **Swyer, G. I. M.:** Biochem. J., **42,** 28, 1948.
41. Idem: Ibid., **42,** 32, 1948.
42. **Thompson, R. T.,** and **Moses, F. E.:** J. Clin. Invest., **27,** 805, 1948.
43. **Waisbreu, B. A.:** Proc. Soc. Exp. Biol. and Med., **58,** 172, 1945.
44. **Wislocki, G. R., Bunting, H.,** and **Dempsey, E. W.:** Am. J. Anat., **81,** 1, 1947.

EXPERIENCES WITH THE DILUTE PROTHROMBIN TIME IN THE DIAGNOSIS OF THROMBO-EMBOLIC DISEASE

By Julius J. Sachs, M.D.
CLINICAL INSTRUCTOR IN MEDICINE
NEW YORK, NEW YORK

(From the Department of Medicine, New York University College of Medicine, and the Third Medical Division, Bellevue Hospital)

Because of the difficulty in the clinical detection of thrombo-embolic disease, there is a need for a laboratory test to confirm the presence of intravascular thrombosis. In 1944, Shapiro[6] suggested that an acceleration of the dilute (12.5%) prothrombin time occurs frequently during the early stage of thrombophlebitis and, or, pulmonary thrombo-embolization. Cotlove and Vorzimer[1] found an accelerated dilute prothrombin time in 4 of 5 non-cardiac patients with thrombo-embolism, and 1 of 8 cardiac patients with thrombo-embolism. The concept of hyperprothrombinemia[6] as a premonitory sign of thrombo-embolization could not be confirmed by Nitsche, Gerarde and Deutsch[5], who correlated an accelerated dilute prothrombin time with an increased plasma fibrinogen level in the majority of determinations. Hurn, Barker and Mann[4], using the dilute prothrombin time, were unable to detect a difference between the control group and the group with thrombosing tendencies. Using barium sulfate treated plasma instead of saline as the diluent, Tuft and Rosenfield[8] were unable to find a significant correlation between thrombo-embolic disease and an accelerated reaction. They found acceleration frequently in early liver disease. Recently, Wiener and Shapiro[9] in a series of human plasmas found no correlation between diluted prothrombin time and fibrinogen concentration, and reiterate the concept of hyperprothrombinemia in the presence of intravascular thrombosis[7].

The following study was undertaken to evaluate the dilute prothrombin time determination as a diagnostic test in patients with thrombo-embolism.

Method. The prothrombin time determinations were performed using the Link-Shapiro modification[6] of the Quick method*. The dilute prothrombin time was determined by a 12.5% dilution of plasma with physiological saline. In our laboratory, in normal subjects, the prothrombin time was 14 to 17 seconds, when undiluted plasma was used, and 33 to 42 seconds with diluted plasma. A dilute prothrombin time of 28½ seconds or less was considered to be accelerated.

Data and Discussion. Prothrombin time determinations were made on patients with a clinical diagnosis of intravascular thrombosis, and on patients with other diseases. In instances where serial determinations were made, the most accelerated value was included in this report. Table 1 summarizes dilute prothrombin time determinations obtained in thrombo-embolic and miscellaneous diseases. In only 36% of patients with intravascular clotting was the dilute prothrombin time found to be accelerated. In a group of miscellaneous diseases without thromboses, 31% of the patients had shortened dilute prothrombin times. The data indicates that there is no significant rela-

* The thromboplastin was kindly furnished by Chilcott Laboratories, the Maltine Company.

tionship between intravascular thrombosis and an accelerated dilute prothrombin time.

The diagnoses of the various cases included in Table 1 are listed in Tables 2, 3, and 4. Three cases (4, 12 and 22) in Table 3 are of particular interest since thrombosis occurred in the presence of a delayed prothrombin time. The markedly prolonged undilute and dilute prothrombin time seen in Patient 12 is especially noteworthy since this patient developed thrombosis of the femoral artery at a prothrombin level which would have been considered therapeutic had the patient been receiving Dicumarol. Two patients in Table 4 had hepatitis, and 2 had cirrhosis of the liver, which is in accord with the findings of Tuft and Rosenfield[8]. In addition, 2 patients with gout and 3 with chronic glomerulonephritis showed accelerated dilute prothrombin values, without clinical evidence of intravascular thrombosis.

Plasma fibrinogen* and dilute prothrombin times were determined simultaneously in a group of patients and the results are plotted in Fig. 1. With few exceptions, the plotted values form a hyperbole and resemble the fibrinogen dilution curves of Deutsch and Gerarde[3]. The majority of accelerated dilute prothrombin times were associ-

TABLE 1.—COMPARISON OF DILUTE PROTHROMBIN TIME IN THROMBO-EMBOLIC AND IN MISCELLANEOUS DISEASES

		Below 29 Sec.		*Above 29 Sec.*	
	Total No.	*No.*	*%*	*No.*	*%*
Thrombo-embolic	56	20*	35.7	36**	64.3
Miscellaneous	64	20***	31.2	44	68.8

* Table 2 ** Table 3 *** Table 4

TABLE 2.—CASES OF THROMBO-EMBOLISM WITH ACCELERATION OF THE DILUTE PROTHROMBIN TIME

		Prothrombin Time (sec.)	
Case	*Diagnosis*	*Whole Plasma*	*12.5% Plasma*
1. Pi.	Phlebothromb.	14½	28½
2. St.	Phlebothromb. after myocard. infarct.	17½	26½
3. D'A.	Phlebothromb., bilat. after myocard. infarct.	16½	28
4. Wi.	Pulm. infarct.	12½	26
5. Ma.	Pulm. infarct.	12	25
6. Go.	Thrombophlebitis, saphenous vein	14	24½
7. La.	Pulm. infarct.	13½	26
8. Ad.	Mult. pulm. emboli, Thrombophleb., poplit. vein	17	28
9. Bo.	Phlebothromb.	14	27½
10. Ki.	Cerebr. thrombosis, Polycythemia	15½	26½
11. Ma.	Thrombophleb., saphenous vein	16½	28½
12. Va.	Thrombophleb., ileofemoral vein, sec. to Ca. of Cervix	15	24½
13. Co.	Thrombosis, dorsalis pedis art., Phlebothromb.	17	26
14. Eg.	Pulm. infarct.	15	26
15. Am.	Embolus, femoral art., Phlebothromb., Myoc. infarct.	15½	28½
16. Bo.	Phlebothromb. bilat.,	13	27½
17. Te.	Thrombophleb., brachial vein	13	24½
18. Bo.	Cerebr. embolus, Subac. bact. endocard.	13½	26
19. Ma.	Phlebothromb.	14½	28½
20. Ho.	Thrombophleb., ileofemoral vein	12½	27½

* Fibrinogen was determined by the method of Cullen and Van Slyke[2].

TABLE 3.—CASES OF THROMBO-EMBOLISM WITH NORMAL OR PROLONGED DILUTE PROTHROMBIN TIME

Case	*Diagnosis*	*Prothrombin Time (sec.)*	
		Whole Plasma	*12.5% Plasma*
1. Ch.	Thrombosis, femoral art.	13½	38½
2. Vo.	Severely thrombosed hemorrhoids	15	38
3. Ba	Phlebothromb., bilat.	16½	37½
4. Fa.	Mult. platelet thrombosis (diagnosed at necropsy)	22½	55½
5. Am.	Phlebothrombosis	17	41
6. Op.	Thrombosis, dorsalis pedis artery	17½	40½
7. Ca.	Phlebothromb.	13½	42
8. Gr.	Phlebothromb.	14	32
9. Bu.	Thrombophleb., ileofemoral vein	14	34
10. He.	Thrombosis, femoral art., Phlebothromb.	14	32½
11. D'Ac.	Thrombophleb., femoral vein	16½	32
12. Ro.	Thrombosis, femoral art.	29½	101½
13. Cr.	Embolus, brachial art.	20	42
14. Ki.	Phlebothromb.	14½	31
15. Mu.	Thromboses, both femoral art. Leukemia	23½	38½
16. Mc.	Thrombophleb., brachial vein	19	41
17. Ho.	Emboli to brach., fem., poplit. arts.	17	35½
18. Kl.	Superfic. thrombophleb.	17	39
19. Si.	Superfic. thrombophleb., Pulm. infarct.	17	42
20. Sb.	Thrombophleb., ileofemoral vein	15	34
21. Ba.	Cerebral embolus, auric. fibrill.	14½	35
22. Ma.	Mult. pulm. infarct.	19½	55
23. El.	Phlebothromb.	16½	36
24. Gr.	Embolus, poplit. art., Phlebothromb.	16½	32
25. Ce.	Pulm. infarct., Phlebothromb.	18	36½
26. Cl	Thrombosis, subclavian vein	14	35
27. Li.	Thrombophleb , ileofemoral vein	14	36
28. Le.	Cerebr. embolus, aur. fibrill.	13½	37½
29. Ka.	Thrombophleb., saphenous and fem. veins	15½	33
30. Ca.	Auric. fibrill. embolus to poplit. art., Phlebothromb.	17	33½
31. Ko.	Thrombophleb., femoral vein	17½	40½
32. Wa.	Phlebothrombosis	15½	34
33. We.	Embolus, poplit. art., Phlebothromb.	18½	34½
34. Pi.	Phlebothromb.	17	39
35. Fl.	Auric. fibrill., Pulm. embolism	17	36½
36. Ra.	Phlebothromb.	17	40½

TABLE 4.—CASES WITHOUT CLINICAL EVIDENCE OF THROMBO-EMBOLISM, WITH ACCELERATION OF THE DILUTE PROTHROMBIN TIME

Case	*Diagnosis*	*Prothrombin Time (sec.)*	
		Whole Plasma	*12.5% Plasma*
1. Ha.	Purpura, nonthrombocytopenic	15½	27
2. Wa.	Bleeding pep. ulcer. Recent transfusions	13	23½
3. Do.	Cholelithiasis	16½	27
4. Sa.	Arterioscl. ht. dis., Cong. ht. failure	14	26½
5. St.	Pneumonia	13	26
6. Bo.	Arterioscl. ht. dis.	13½	24
7. Ap.	Arterioscl. ht. dis., Cong. ht. failure	15½	28½
8. Mo.	Pulm. fibrosis and emphysema	15	26½
9. Jo.	Thyroid adenoma, Diab. mellitus, Aspir. pneum.	15	17½
10. Fo.	Cirrhosis of liver	12	26½
11. Tr.	Ac. follic. tonsillitis	13½	28
12. Fe.	Chr. glomerulonephr.	15½	22½
13. Mu.	Cirrhosis of liver	14	24
14. Go.	Infectious hepatitis	13	26½
15. O'L.	Acute nasopharyngitis	13½	27
16. St.	Chr. glomerulonephr.	16	28½
17. Ja.	Gout	16½	25½
18. Po.	Gout	11½	27
19. Ma.	Infectious hepatitis	12	24½
20. Ho.	Chr. glomerulonephr.	13½	26

ated with an elevated plasma fibrinogen level.

In an attempt to determine whether an accelerated dilute prothrombin time represents hyperprothrombinemia, a comparison was made between the amount of Dicumarol required to maintain therapeutic levels in patients with and without an accelerated dilute prothrombin time. If the concept of hyperprothrombinemia is valid, one might expect patients with an accelerated dilute prothrombin time to require larger doses of Dicumarol. This was not found. In 11 patients with an accelerated dilute prothrombin time, the average Dicumarol dose for 14 days was 1181 mg. In 23 patients, with normal dilute prothrombin time, the average dose for 14 days was 1117 mg. Although individual differences in rates of absorption and sensitivity may have influenced the results, it seems such probably has no more specificity than the erythrocyte sedimentation rate.

The fact that patients with accelerated and normal dilute prothrombin times require similar doses of Dicumarol for maintainence of therapeutic levels suggests that an accelerated dilute prothrombin time is not indicative of hyperprothrombinemia.

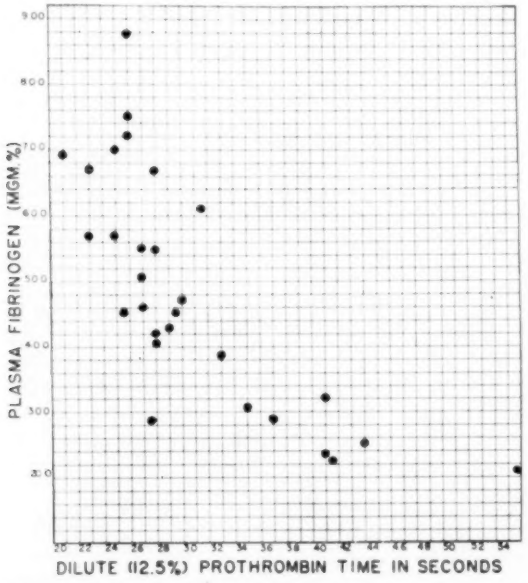


FIG. 1.—Effect of plasma fibrinogen on the dilute prothrombin time.

Summary. In our experience, an accelerated dilute prothrombin time did not reflect the presence of intravascular thrombosis. The test was found to be accelerated in only one third of patients with intravascular clotting, as well as in one third of patients without thromboses.

The acceleration appears to be related to the fibrinogen level and as

REFERENCES

1. **Cotlove, E.**, and **Vorzimer, J. J.**: Ann. Int. Med., **24**, 648, 1946.
2. **Cullen, G. E.**, and **Van Slyke, D. D.**: J. Biol. Chem., **41**, 587, 1920.
3. **Deutsch, H. F.**, and **Gerarde, H. W.**: Ibid., **166**, 381, 1946.
4. **Hurn, M., Barker, N. W.**, and **Mann, F. D.**: Am. J. Clin. Path., **17**, 704, 1947.
5. **Nitsche, G. A., Jr., Gerarde, H. W.**, and **Deutsch, H. F.**: J. Lab. and Clin. Med., **32**, 410, 1947.
6. **Shapiro, S.**: Exp. Med. and Surg., **2**, 103, 1944.
7. **Shapiro, S.**, and **Weiner, M.**: Coagulation, Thrombosis and Dicumarol, New York, Brooklyn Medical Press, 1949.
8. **Tuft, H. S.**, and **Rosenfield, R. E.**: Am. J. Clin. Path., **17**, 704, 1947.
9. **Weiner, M.**, and **Shapiro, S.**: Blood, **4**, 977, 1949.

THE DIAGNOSTIC SIGNIFICANCE OF URINARY PEPSINOGEN EXCRETION IN DISEASES OF THE UPPER GASTROINTESTINAL TRACT*

By Henry D. Janowitz, M.D.
RESEARCH ASSISTANT IN MEDICINE

Milton H. Levy, M.D.
RESEARCH ASSISTANT IN GASTROENTEROLOGY

AND

Franklin Hollander, Ph.D.
HEAD, GASTROENTEROLOGY RESEARCH LABORATORY
NEW YORK, NEW YORK

(From the Gastroenterology Research Laboratory, The Mount Sinai Hospital)

Urinary pepsinogen (uropepsin) is the proteolytic enzyme of urine which is active in the acid range of pH. Although the existence of this enzyme has been known since 1861[2], interest in the subject has only recently increased—possibly because of the development of more accurate methods of determining pepsin, but certainly stimulated by the review of this subject by Bucher[3].

Some aspects of the physiological behaviour of uropepsin seem clear. The enzyme is stable in alkaline urine and seems to be identical with gastric pepsinogen. It is derived from the glands of the gastric mucus membrane, since it is absent from the urine of gastrectomized animals[4,6], and also of subjects with the achylia gastrica of pernicious anemia[5,11]. This proenzyme, formed in the peptic cell, is evidently partitioned into exocrine and endocrine fractions. The major portion is secreted in the inactive form into the gastric lumen, where it is converted to active pepsin in the presence of HCl. In the latter form it cannot undergo gastrointestinal absorption, as evidenced by the observation that the feeding of pepsin to men and animals does not increase their urinary excretion of the enzyme[9]. The smaller portion of pepsinogen diffuses from the peptic cells into the blood stream by way of the tissue fluid, and is excreted in the urine without being activated. Gastric analyses were performed on only a few of the patients in this study. We have, however, just completed another systematic study in this laboratory, and the evidence obtained[8] supports the concept that uropepsin excretion increases with the level of gastric secretory activity.

Clinical studies by Farnsworth, Speer, and Alt, in patients with pernicious anemia[5], by I. A. Mirsky and collaborators in subjects with peptic ulcer[11], as well as the older work of Gottlieb[7], have indicated that the determination of uropepsin may have diagnostic significance. Accordingly, the present study was undertaken to obtain evidence for assessing the value of this determination under standardized conditions, in patients with disorders of the upper gastrointestinal tract.

* Preliminary report presented before the 22nd Graduate Fortnight of the New York Academy of Medicine and published in the Bull. N. Y. Acad. Med., **26**, 281, 1950.

Methods. Urinary pepsinogen was measured by I. A. Mirsky's modification[9] of the Anson-Mirsky-Bucher method for pepsin, using the lyophilized bovine hemoglobin powder as substrate recently reported from this Laboratory[10]. The amount of proteolysis is measured colorimetrically in terms of tyrosine liberated. All determinations were run in duplicate, and paralleled with a blank of urine inactivated with trichloracetic acid. In keeping with the notation developed originally by Anson and Mirsky[1], a practical unit of uropepsin activity may be defined as the amount of enzyme which releases $1x10^{-4}$ mEq. of tyrosine from the substrate under their standardized conditions.

Urines were collected for periods of 3 to 12 hours, either with the subject in the fasting resting state, or 5 to 8 hours postprandially. The results of the enzyme measurements were expressed as the average number of units of uropepsin excreted per hour (units/hr.) throughout the period of collection.

Clinical Material. Ninety-nine subjects were investigated in this study. They fell into 6 clinical categories, characterized as follows: 1) A control group, without gastrointestinal complaints or demonstrable disease; 2) patients with duodenal or marginal ulceration following subtotal gastrectomy: clinically active, and confirmed either by the roentgen demonstration of an ulcer niche, or by surgical resection; 3) patients with benign gastric ulcer proven by histological examination following gastric resection; 4) individuals with malignant gastric neoplasms; 5) subjects with the achylia gastrica of Addisonian pernicious anemia; 6) a patient with a total gastrectomy. Renal function, as judged by routine urinalysis and blood urea nitrogen, was normal in all subjects.

Results. The urinary pepsinogen excretion per hour for each of these clinical categories is listed in Table 1, where the range for each group and the mean value is given. The frequency distribution of the uropepsin values is given in Table 2 for each of the clinical groups studied.

1. *Control group.* This comprised 44 individuals, both normal subjects from the laboratory staff and convalescent patients from the hospital wards. These subjects had either no gastrointestinal complaints or only minor ones with no demonstrable gastrointestinal disease. They excreted between 0 and 136 units per hr., the average value being 47. It will be seen from Table 2 that 95% of this group excreted less than 100 units per hr.

2. *Duodenal and stomal ulcer group.* This comprised 35 subjects: 29 with duodenal, and 6 with jejunal ulceration. All were clinically active, and the ulcer was demonstrated either as a niche by Roentgen-ray, or by surgical resection. These patients excreted between 114 and 373 units per hr., the average being 180. Thus, on the average, the duodenal and marginal ulcer group excreted 4 times as much uropepsin as did the control group. Despite the overlap of the ranges of these 2 groups, the difference between their means is statistically significant ($t = 11.3$, $n = 77$, $P < .0001$).

3. *Gastric ulcer.* Five subjects with proven benign gastric ulcers excreted between 43 and 125 units per hr., the average being 80. This group is small, but apparently falls within the values of the control group.

4. *Gastric neoplasms.* Seven subjects were studied in this category: 6 with carcinoma, one with a leiomyosarcoma. The range in this group was 0–112 units, the average 53. Again, although small, these values fall entirely within the range for the control group.

5. *Pernicious anemia.* Seven subjects with Addisonian pernicious anemia were studied; 4 of them were clinically in remission, and 3 in relapse. All had a histamine refractory achlorhydria, and the absence of pepsin in the gastric juice. All excreted no uropepsin.

6. *Total gastrectomy.* This patient was incapable of elaborating pepsin-

TABLE 1.—URINARY PEPSINOGEN EXCRETION IN UNITS PER HOUR

Clinical Category	*No. of Cases*	*Range*	*Mean*	*S. D. Mean*
a. Control	44	0–136	47	(± 6)
b. Duodenal or stomal ulcer	35	114–373	180	(± 12)
c. Gastric ulcer	5	43–125	80	
d. Gastric neoplasm	7	0–112	53	
e. Pernicious anemia	7		0	
f. Total gastrectomy	1		0	

TABLE 2.—FREQUENCY DISTRIBUTION—UROPEPSIN EXCRETION PER HOUR

Uropepsin Excretion Units/hour	*Clinical Categories*					
	Control	*Duodenal and Stomal Ulcer*	*Gastric Ulcer*	*Gastric Neoplasm*	*P.A.*	*Total Gastrectomy*
0	5			1	7	1
1–19	5					
20–39	8			2		
40–59	13		1	2		
60–79	6		2	1		
80–99	5					
100–119		4	1	1		
120–139	2	8	1			
140–159		8				
160–179		1				
180–199		3				
200–219		3				
220–239		1				
240–259		3				
260–279		1				
280–299						
300–366		3				
No. of Cases	44	35	5	7	7	1

ogen, and this was reflected in his failure to excrete uropepsin.

Discussion. These results are in keeping with the reports of I. A. Mirsky and his collaborators[9] and indicate clearly that our subjects with active duodenal or stomal ulceration excreted, on the average, 4 times as much urinary pepsinogen as did the group without such ulceration. The combined groups of gastric ulcers and gastric neoplasms is admittedly small, but these patients all excreted uropepsinogen in amounts similar to the control groups.

In view of the relationship shown by us to exist between gastric secretory activity and uropepsin output in the resting state, these observations are consistent with the known hyper-secretory activity of the duodenal ulcer group, and the normal or low secretory activity of the gastric ulcer patients. The inability of the patients with Addisonian pernicious anemia to elaborate pepsinogen is reflected in their failure to excrete any uropepsin. Our findings in this regard are similar to the previous observations of Farnsworth *et al.*[5], and Mirsky *et al.*[9]. Two patients with megaloblastic anemia of non-tropical sprue excreted normal amounts of uropepsin, however.

Although a urine collection is con-

siderably easier and more convenient than the routine gastric intubation or barium meal, it appears that the uropepsinogen criterion serves only to exclude or include duodenal ulcer and does not furnish the anatomic evidence of the roentgen examination. However, in the specific situation of active gastrointestinal bleeding, where the conventional diagnostic work-up must be temporarily held in abeyance, this method can furnish a valuable clue to the nature of the underlying disease, during this difficult period of management. Also, in investigation of subjects with anastomotic operations on the upper gastrointestinal tract, where gastric juice uncontaminated by intestinal fluid cannot usually be obtained, uropepsinogen determination may be useful in establishing the presence or absence of active gastric secretory mucus membrane.

These considerations are balanced, however, by the fact that the accurate determination of peptic activity does not lend itself to performance in a routine laboratory at present. From the investigative point of view, however, this approach appears to be valuable as a method for following gastric pepsin secretory activity over long periods of time, with considerably more ease than the intubation technique.

Summary. The urinary pepsinogen excretion of 99 subjects was determined. Thirty-five subjects with active duodenal or stomal ulceration excreted, on the average, 4 times as much uropepsin as did the 44 control subjects. A group of 12 patients with gastric neoplasms and gastric ulcers excreted normal amounts of uropepsin. Seven patients with pernicious anemia, and 1 with a total gastrectomy excreted no uropepsin.

The determination of urinary pepsinogen excretion appears to have considerable value as an addition to the conventional diagnostic approach to disorders of the upper gastrointestinal tract. This is especially true in the bleeding patient, and in the patient with a neutralization anacidity following a gastrointestinal anastomosis.

REFERENCES

1. **Anson, M. L.,** and **Mirsky, A. E.**: J. Gen. Physiol., **16**, 59, 1932.
2. **Brucke, E.**: Sitzber. Akad. Wiss. Wien. Math.-naturw. Klasse, **43**, 618, 1861.
3. **Bucher, G. R.**: Gastroenterology, **8**, 627, 1947.
4. **Bucher, G. R.,** and **Ivy, A. C.**: Am. J. Physiol., **150**, 415, 1947.
5. **Farnsworth, E. B., Speer, E.,** and **Alt, H. L.**: J. Lab. and Clin. Med., **31**, 1025, 1946.
6. **Frouin, A.**: Compt. rend. soc. biol., **56**, 204, 1904.
7. **Gottlieb, E.**: Skand. Arch. Physiol., **46**, 1, 1924.
8. **Janowitz, H. D.,** and **Hollander, F.**: Fed. Proc., **9**, 67, 1950.
9. **Mirsky, I. A., Block, S., Osher, S.,** and **Broh-Kahn, R. H.**: J. Clin. Invest., **27**, 818, 1948.
10. **Orringer, D., Lauber, F. U.,** and **Hollander, F.**: Science, **111**, 88, 1950.
11. **Podore, C. J., Broh-Kahn, R. H.,** and **Mirsky, I. A.**: J. Clin. Invest., **27**, 834, 1948.

PROGRESS
OF
MEDICAL SCIENCE

GYNECOLOGY AND OBSTETRICS

UNDER THE CHARGE OF

CARL BACHMAN, M.D.

PROFESSOR OF OBSTETRICS AND GYNECOLOGY AT THE HOSPITAL OF THE UNIVERSITY OF PENNSYLVANIA

AND

FRANK B. BLOCK, M.D.

JEWISH HOSPITAL OF PHILADELPHIA

PHILADELPHIA, PENNSYLVANIA

THE FEMALE URETHRA

By FRANK B. BLOCK, M.D.

SURGEON, JEWISH HOSPITAL

PHILADELPHIA, PENNSYLVANIA

THE female urethra is about 1½ inches in length, being much shorter than the canal in the male, and embryologically corresponds to the portion of the latter that lies between the internal urethral orifice and the openings of the ejaculatory ducts. Being so relatively small an organ, it may not receive the attention it deserves when pelvic disease is being diagnosed or treated.

PARAURETHRAL DUCTS. There has not been much in the literature recently concerning its anatomy, but a detailed study of the paraurethral or Skene's ducts has been made by Huffman[9], who states that there has been some question relative to the presence of urethral glands other than Skene's ducts. The material for his study was obtained from 11 necropsies. From each a mass of tissue was excised which contained the entire urethra and the surrounding structures lying beneath the pubic arch, the lower portion of the bladder, and the anterior vaginal wall. After fixation each mass was cut to a size suitable for histologic preparation. When so trimmed, each block consisted of the urethra, the periurethral tissues, and the intact suburethral vaginal mucosa. These blocks were then cut transversely into several serial blocks and, subsequently, each block was serially sectioned at 10 micra. Every twenty-fifth section was stained with hematoxylin and eosin. When wax models were to be made, these sections were placed under a projecting microscope and enlarged. The enlarged images were transferred onto paper, the outlines of the desired structures were traced, and they were transferred from the paper onto wax plates whose thickness was such as to maintain correct longitudinal proportions. The serial wax plates were subsequently annealed to form models.

During vaginal examinations duct-like openings just outside and dorsal to the urethral meatus are frequently noted. Occasionally these are the

mouths of minor vestibular glands; more often they are entrances of paraurethral ducts. They are usually seen in multiparas, and their locations may result from distortion which was a concomitant of labor and which produced a flattening of the urethral eminence with eversion of the distal urethral mucosa. In women who have not borne children, the urethral labia are ordinarily well formed, the urethral mucosa is not everted, and the openings of the paraurethral ducts are usually not visible until the urethral labia are separated and the outermost mucosa exposed. In all specimens the greatest number of paraurethral ducts empty into the distal one-third of the urethral canal. There is a definite tendency for the mouths of the ducts to be in 4 groups, with 2 groups on each side (that is, on each side there is a ventrolateral group and a dorsolateral group). When larger tubules are present they are usually in the dorsolateral location. In addition to these major groups there is, however, a considerable number of ducts opening into the lateral, the dorsal, and a few in the ventral urethral walls. After leaving the urethral canal these tubules turn cephalad and promptly divide into small branches. In the material studied the presence of 2 large lateral ducts as described by Skene was the exception rather than the rule, and the widespread branching of the terminal divisions was more marked than Skene described. In but 2 of 11 specimens could 2 larger tubules, one on each side of the urethra, be followed for any distance. Except for their greater diameter and longer length, these tubules resembled the other paraurethral ducts seen in the same specimen and in other specimens; they were lined by the same type of epithelium and terminated in tubular glands just as the other ducts described herewith. The least number of ducts found in any of the urethras examined was 6, the most 31.

URETHRAL CARUNCLE. Perhaps the best known lesion of the female urethra is the urethral caruncle concerning which there has been much controversy as to its etiology, pathology and treatment. A study of 120 cases of this lesion has been made by Palmer, Emmett and McDonald[11] of the Mayo Clinic who state that the symptoms are out of all proportion to the size of the lesion. The most prominent symptom is usually pain which has been described as "scalding," "stabbing," "shooting," "cutting," and "burning." It has been variously described as being referred to genital organs, hypogastrium, perineum, kidneys, rectum, bladder, lumbosacroiliac region, and soles and heels of feet. The pain is said to be produced by micturition, sitting, walking, coitus, touch, menstruation, and friction from clothing, napkins, and so forth. The pain is occasionally so great that the patient puts micturition off until the urge becomes unbearable, leading to dribbling, and incontinence. Occasionally the discomfort is so great that it leads to neurasthenia, loss of weight, insomnia, despondency, melancholia, and other mental disturbances. On the other hand some caruncles are painless. Another prominent symptom of the urethral caruncle is bleeding. The blood is usually seen at the beginning or end of urination, following coitus, friction from clothes or pads, following trauma, and on toilet tissue. It is usually spotty—that is, there are only a few drops at a time but occasionally it may be so profuse that the patient thinks she is menstruating irregularly. The first noticeable finding may be a small tumor mass and this may be the only symptom which brings the patient to the physician. Other symptoms are division of the urinary stream, urinary frequency, nocturia, and pruritus. Any one or all of the foregoing symptoms may be

present. The gross appearance of urethral caruncle has been described as a bright red growth like a miniature "cock's comb" or like a very small raspberry springing from the lower margin of the meatus urinarius. The size may vary from that of a pinhead to that of a walnut, but the caruncle is usually the size of a pea. It is usually single but may be multiple. The term "urethral caruncle" has become a clinical wastebasket into which most lesions of the urethra are placed. There are almost as many terms for describing urethral caruncles as there are articles on the subject. There has been a tendency for physicians to remove urethral growths as an office procedure and simply call them "caruncles" and forego any pathologic study. This has led in many cases to what is called a "caruncle becoming malignant" because at a later date a biopsy revealed "carcinomatous changes". To be certain of the diagnosis, microscopic examination of the tissue is imperative. Urethral caruncle is a clinical rather than a pathologic term. However, the condition is by no means rare and therefore constitutes a rather common diagnosis for the pathologist. The histologic structure is not constant, a fact which explains the diversity of pathologic terms used to designate urethral caruncles as papillary angioma, vascular polyp, capillary angioma, urethral hemorrhoids, adenoma, granuloma, and others. The marked vascularity and inflammatory reaction are most commonly noted and mentioned. The controversy as to whether the urethral caruncle is always benign or potentially malignant still exists. No one treatment is acceptable or applicable in all cases. The incidence of recurrence is high if the patients are followed long and closely enough. In this series of 120 cases, nearly two-thirds of the patients who answered follow-up letters had recurrences of the caruncle regardless of the method used. The number of recurrences varied from 1 to 9. With this high rate of recurrence, the patient should be warned that recurrence is extremely likely and that further treatment may be necessary. The main purpose of the treatment offered is symptomatic relief. This should be secured by the simplest and most suitable treatment, depending on the nature of the lesion.

An analysis of two series of urethral caruncle made by Walther[16], totaling 100 cases, has shown 5 instances of unsuspected carcinoma and 2 instances of precancerous lesions in the 47 cases in which pathologic study was carried out. A comparative consideration of the clinical aspects of urethral caruncle and urethral carcinoma makes clear that little reliance can be placed upon diagnosis by purely clinical means, because of the similarities between these conditions. Since histologic examination is imperative in every instance of presumable urethral caruncle, in order to exclude carcinoma, it is important that a method of treatment be employed which permits the securing of a specimen suitable for microscopic study. He describes his method of electrosurgical excision which meets this criterion and which also is simple, can be quickly performed, removes the growth completely, and results in minimal scar tissue. After dilatation has been accomplished, a traction suture is passed deeply through the base of the caruncle, which is thus brought into view. An electric cutting loop, just large enough to encompass the tumor, is threaded over the traction suture and is brought firmly into contact with the base of the lesion. The cutting current is then applied until the growth with its base has been completely removed. Bleeding is controlled as necessary with the coagulation current. It has been as effective in malignant as in benign growth in his hands. Whatever method of treatment is adopted, dilata-

tion of the urethra is necessary after operation. In the few cases in which dilatation is unsatisfactory, meatotomy can be carried out with a small electrocautery knife. If urinary tract or secondary infection is present before operation, forced fluids, sulfa drugs, applications of silver nitrate, instillations of neosilvol, and hot sitz baths are used according to the indications. The same methods are used if these complications develop after operation.

Carcinoma. Primary cancer of the female urethra is not a common lesion, as evidenced by the fact that only 17 such cases were seen over a period of 12 years on the Urological Service of the New York State Institute for the Study of Malignant Disease. In reporting these cases Watson[17] states that the diagnosis of urethral carcinoma in the female should not be difficult. In its simple papillomatous form it must be distinguished from caruncle, polyp, benign papilloma, and leukoplakia with hypertrophy. Fortunately, most urethral growths protrude either slightly or considerably from the meatus. Fortunately, too, the female urethra can be readily palpated along its entire length either about a small sound or with its walls approximated. All tissue removed from the urethra as lumps, polyps, or caruncles should be examined for malignancy, and, when any appreciable thickening or induration is felt along the urethra, the base of the lesion should be inspected frequently through the endoscope. If any proliferation or unreasonably delayed healing is encountered, a 2nd or even a 3rd piece of tissue should be removed for biopsy. In 5 cases in this series the first section obtained, though satisfactory in size and quality when studied microscopically showed in 1) infected granulation tissue, 2) urethral caruncle, 3) infectious papilloma, 4) squamous cell proliferations, 5) papilloma of the urethra. In each of these instances subsequent sections taken 1 week to 2 months later showed a true mucous membrane epithelioma or carcinoma of the urethra. This point is stressed because it is seen that in nearly 30% of the cases a second section was necessary to obtain an accurate and early diagnosis. The treatment of the patients in this series has been in the form of 1) surgery (electro coagulation, and more recently cutting current excision), 2) high power Roentgen-rays, 3) radium emanation implantation, and various combinations of these methods. The larger tumors protruding from and involving the entire circumference of the urethra and at times extending half way and sometimes farther up the urethra can successfully be excised by using the cutting current. With a sound, or preferably a catheter, in the urethra the area beneath the clitoris is incised and the incision is then carried down on either side just beneath the labia minora. When necessary, however, this incision may be made to include the clitoris and extend up to the symphysis above and to the pubic rami on either side. The tumor is then drawn forward by traction and the urethra is cut across, leaving the internal sphincter intact. If necessary further tissue beneath the urethra may be included so that the growth is removed en masse. Bleeding is usually not disturbing, most of it being controlled by the cautery current and rarely by transfixion sutures. Several linen sutures are then placed across from the base of each labium minus, stretching the mucosa and submucosa to bridge the denuded surface. The cut end of the urethra is allowed to protrude along the line of suture, and it is fixed on either side with small sutures passing through the periurethral structures, and leaving the stump to protrude slightly. Stricture of the meatus appears less likely to form if there is a short end of urethra left free and its mucosa not pierced with

sutures. Subsequent to excision of the tumor deep Roentgen-ray treatments have been given, usually 2 to the anterior pelvis and 2 to the posterior pelvis. When there are palpable glands present in the groins these areas are also treated by Roentgen-rays. When the urethral growth is not massive in type, and its lateral margins not greatly invading toward the labia, the bulk of the tumor has been removed by electro coagulation or by loop resection and its base planted with gold seeds of radium emanation, supplemented by deep Roentgen-ray to the pelvis and also to the groins when palpably enlarged glands are present. In the group of smaller carcinomata involving the base of the urethra, definitely indurating more toward the internal sphincter, and with the hard ring-like enlargement about the meatus, the implantation of radium emanation seeds supplemented by deep Roentgen-rays has been the method of choice.

In the series of 22 cases reported by Auer[1], the youngest patient was 39 and the oldest 75, the average age being 54; this high average being in accord with the statistics of other authors. Treatment of his cases has consisted of local excision or local radiation, with small, well-screened doses of radium. Radium is best used in the form of emanations, in either gold or platinum seeds. If this form of radiation is unavailable, 25 to 50 mg. of radium element, screened in the equivalent of 1.25 mm. of platinum, surrounded by rubber sewed into the urethral canal at the site of the lesion, should be used. He has employed from 400 mg. hours to 4100 mg. hours in the local treatment of urethral carcinoma, the amount depending entirely on the size of the local lesion. No arbitrary dosage of radium can be prescribed, as radium dosage is entirely a matter of experience. Despite the large amount of tissue damage resulting from some of the radiation, only 2 patients had incontinence of urine. In certain instances radical surgical removal of the regional lymphatics, both above and below the inguinal ligament (Basset operation) was done in addition or in combination with the local treatment. He believes that this operation, combined with local radiation, is the treatment of choice in all early and moderately advanced cases of cancer of the female urethra, if in the judgment of the operator the patient is a fairly good operative risk. The small size of a local lesion that has proved to be cancer by microscopic examination should never influence one toward conservatism in the treatment of this condition, for metastases have been found in the regional lymph nodes with an extremely small primary lesion.

According to Pomeroy[12] interstitial irradiation, using either gold radium implants or removable platinum needles containing radium element, is a satisfactory method of treating carcinoma of female urethra. The treatment of regional lymph nodes presents a problem fully as important as the problem presented by local lesion. If a local tumor disappears under irradiation, the lymph nodes may be treated by surgical excision or by irradiation. Occasionally, nodes that are not palpable may be kept under close observation and no treatment employed unless definite enlargement is detected.

In the opinion of Hess[8] cancer of the female urethra is a preventable disease if the condition of granular urethritis is recognized and adequately treated. Unfortunately, little attention is paid to the urethra by women or their physicians even after symptoms develop. All hyperplastic conditions of the female urethral mucosa must be considered malignant until proven otherwise by biopsy. The majority of these conditions—mucosal hypertrophy with eversion, the external and internal caruncle, and the suspicious malignancy—usually occur in women between 40 and 70

years old, with a history of genital infection of long standing, with or without injury to the birth canal. The operative technique is very simple and may cure. The percentage of malignancies in these mucosal hyperplasias is much larger than ordinarily considered, approximately 33%. With glandular metastasis, which is usually late, the prognosis is bad. With extensive infiltration along the anterior vaginal wall, the prognosis is better if transplantation of the ureters to skin or bowel is followed by total cystectomy and the removal of a large flap of the anterior vaginal wall. Most of these cases do not respond to radium or Roentgen-ray therapy alone. He recommends removal of the lesion.

Posterior Urethritis and Stricture. It is the belief of Folsom and O'Brien[6a] that many women have active inflammatory processes in the glands of the posterior urethra. This process may be acute or chronic and in many cases a stricture is present. The two processes, that is, the severe papillary or granular posterior urethritis and the stricture, go hand in hand to produce a pathologic clinical entity which can and does cause many women untold suffering for years with either a continuous or a frequently recurring bladder irritation characterized by frequency and burning. These symptoms vary widely in different women; some are merely annoyed by occasional attacks of a mild degree, while others may have almost a constant frequency and burning which makes them miserable and, as one can well imagine, they get extremely nervous from this constant assault on their nervous systems. In their experience, of all women who come for any urologic complaint, at least 90% have this bladder irritation as one of the presenting symptoms, if not the main one. Of this group, 90% have as the sole cause of this troublesome symptom complex an inflammatory urethritis. In spite of this, the urine in these cases is usually free from pus. Many women have been advised to have a suspension of the uterus to lift it up away from where it was pressing on the bladder. The surgeon or gynecologist told them that the uterus had fallen forward so that it was pressing on the bladder and that this was the cause of their bladder irritation. In their judgment, this is highly fallacious, because they have seen so many women who have had suspensions done for the relief of bladder irritation and then continued to have the same trouble until this urethral lesion was properly treated. Repairs of cystoceles have also been done for the relief of bladder irritation. Here, too, they believe that if one has a definite reason for doing a cystocele operation it should certainly be done; but if one is doing it primarily to relieve the woman of bladder irritation, one should first have the posterior urethra studied very carefully before operating; for in the great majority of such cases one will fail to get a result, not because the operation was poorly done but because the real cause of the woman's trouble was not the cystocele but a chronic inflammatory posterior urethra. The next symptom produced by this inflammatory posterior urethritis is pain. This pain is a referred pain manifesting itself in various locations. These pains are, as a rule, dull and aching in character, but at times they may be so closely similar to renal colic in location and severity that it may be difficult for one to be sure one is not confronted with a true renal pain. The most frequent sites for this referred pain are either in the right or the left iliac region, low down, the lumbosacral region of the back, either loin, where if the pain is severe it may be, and has frequently been, mistaken for renal or ureteral disorder, or the inner side of the thighs. They have paraphrased one of Richard Cabot's famous dicta thus: "Any pain within 2

feet of the female urethra for which one cannot find an adequate explanation should be suspected of coming from the female urethra." Their records are literally filled with women who have had, through the years, just 2 definite symptoms, bladder irritation and pain, in one of these locations. They have had from 1 to 6 or 8 abdominal operations with absolutely no relief from either the bladder irritation or the pain. In a tremendous majority, they found these women to be suffering from a chronic papillary or granular posterior urethritis, and the correction of this trouble has almost uniformly given them relief, not alone from one, but from both complaints. The foundation work of the treatment of this troublesome pathologic condition is urethral dilation. This should be done twice a week regularly, beginning with the size sound that will stretch but not tear the urethral mucosa. Then the size of the sound should be gradually increased until a 30 or 32 F. will enter easily. After each dilation they instil 10 to 14 cc. of 0.25% solution of strong protein silver. When the urethra has been dilated for about 6 weeks, the next thing to do is to fulgurate some of the pathologic tissue in the posterior urethra. After this fulguration, the patient is allowed to rest for 2 weeks. Then the dilations are resumed and given for a period of 6 weeks to 2 months, when another fulguration may be done. This routine is followed until the patient is relieved and there is no further evidence of pathologic change present in the posterior urethra. In some cases that are refractory to treatment, one should never lose sight of the fact that there is a very intimate lymphatic connection between the cervix uteri and the posterior urethra and trigone. Hence these patients may be and often are helped by clearing up an old endocervicitis, which may have been playing a part in the perpetuation of the urethral infection.

It has been Stevens's[14] practice to calibrate the urethra of every female patient with symptoms suggestive of a pathologic condition of the urinary tract. The results of this routine procedure, together with the marked improvement following treatment by dilation or meatotomy in those cases in which a diagnosis of stricture was made, have confirmed the opinion that congenital or acquired strictures of the female urethra are of frequent occurrence. In a study of 1227 women patients with urinary disturbances he found that strictures were partly or wholly responsible for the symptoms in 458, or 37%. A large majority, about 85.6%, were found at the external meatus. A ring or thin circular band of tissue was seen at this location on withdrawal of a bulb bougie in at least one half of these cases. This band is usually higher at the inferior margin of the meatus and if more extensive could be classified as a diaphragm or valve. Practically all congenital strictures are of this type, and it does not seem improbable that many if not all of these are, like diaphragms, due to a persistent malformation of the cloacal membrane. Strictures in the canal and at the internal urethral orifice are almost always acquired. The urethras of 118 adult females who had never suffered from urinary disturbances have been calibrated and the average size was found to be F .26. The average size in 174 strictures, on the other hand, was F 21.45. Ninety-eight per cent of his patients complained of frequent urination and 60.3% of nocturia. Congenital strictures are probably responsible for many of the cases of enuresis so common in children. The diagnosis is simple if the possibility of their occurrence is remembered and the urethra calibrated with olive tip bougies. A predisposition to infection is present whenever there

is obstruction, and congenital strictures are probably important etiologic factors in the cystitis and pyelitis so frequently encountered in female infants and children. Although many strictures at the external orifice of the female urethra yield readily to dilation, and temporary improvement is common, meatotomy is the procedure of choice for obstruction at this location. It is preferable to bring the skin and mucous membrane together on both sides of the meatus with fine catgut following incision. This will result in less postoperative bleeding and prompt healing. Further passage of dilators is seldom required.

Prolapse of the Urethra, according to Berry and Green[3], is found in females of all ages but chiefly in young girls. In most instances, it develops suddenly, although it may be slow and progressive. The symptoms are variable but there is usually irritation or burning in the region of the urinary meatus with the presence of a small red mass. This protrusion varies in color with the degree of strangulation from a deep red to blue, with or without ulceration or even gangrene. In differentiating this condition from other lesions, it should be noted that in urethral prolapse the tumor surrounds the entire circumference of the meatus which can easily be verified by placing a catheter in the urethra. In mild cases, wet compresses may cause replacement. Many surgical measures have been advocated for the severe cases. The authors recommend the fulgurating current to amputate the prolapsed, strangulated mucosa, followed by the use of an indwelling catheter for 2 or 3 days. After this, the urethra should be dilated occasionally to prevent meatal stricture.

Diverticulum. It has often been assumed that urethral diverticula result from infection in a urethral gland, most probably of gonorrheal origin, but after dissecting out many of the diverticula in his series of 71 cases at the Mayo Clinic, Counseller[4] believes that they originate as congenital defects of the urethra and are subsequently influenced by trauma and nonspecific infections. They are analogous to diverticula of the intestinal tract or bladder in that they do not cause trouble unless they are unable to evacuate their contents completely. Any diverticulum that cannot empty itself will become infected and produce an abscess. Diverticula of the urethra never contain all the normal layers of the urethra. The sac has a mucosal lining; this, however, may be necrotic or ulcerative and difficult to identify. Further evidence that urethral diverticula are congenital in origin is found in the fact that some are of a large size, perhaps as long as the urethra and 1 cm. in diameter and yet there is no history that an abscess has been present in the region. From many of these urine can be expressed freely and it usually has an ammoniacal odor. The cardinal symptoms were pain and frequency of urination, leakage of urine, dyspareunia and, finally, a vaginal mass. The urinary symptoms may be mild to severe. Often the urethra does not show any local inflammation at all but the voided specimen will contain large amounts of pus even though contamination from the vagina is excluded. The catheterized urine may be entirely negative and this fact may lead to a mistaken diagnosis. Vaginal examination with careful palpation of the anterior vaginal wall under the entire urethral region may reveal some induration or a definite mass. Pressure on the mass will cause foul-smelling urine or pus to exude from the external urethral orifice unless the orifice of the diverticulum has been occluded by the inflammatory process. If there is only induration, the diverticulum can be more easily identified if a urethroscope is placed in the urethra and then the urethra is palpated over it. Cystoscopy

is not always successful in identifying the diverticulum. It has been his experience that any local treatment, such as massage, dilatation, or instillations, is of no value when a real diverticulum exists. Transurethral incision of very small diverticula for more adequate drainage may be useful. The best treatment, however, is complete surgical excision of the diverticulum and repair of the urethra. This can be accomplished in practically all cases except the few in which abscesses are present. If the diverticulum is firmly adherent to the urethra and at all inflammatory, all of the sac should be excised except that small segment along the urethra. The orifice in the urethra can be closed with interrupted fine catgut sutures. This region should be reinforced by a running suture which closes the fascia over the entire urethra including the defect. A catheter is maintained in the urethra for only 4 to 5 days; the patient then is advised to go to the bathroom and void. Subsequent catheterizations are inadvisable.

Trafton[15] reports 6 cases of diverticula that were observed at the Lahey Clinic. He states there is no characteristic symptomatology but a urethral diverticulum should be suspected in any patient with a swelling in the anterior vaginal wall or about the urethra. Direct inquiry should be made regarding such a swelling in any patient with recurrent or persistent bladder disturbances. Surgical excision is the treatment of choice in most cases, although good results have been obtained by conservative measures. Small, wide-necked diverticula are particularly amenable to periodic urethral dilatation which may be followed by instillation of silver nitrate, 1:250 dilution. A diverticulum with stone may occasionally be treated successfully by intraurethral manipulation followed by urethral treatment. Large diverticula, in particular those with small intraurethral communications, should be excised. With the patient in the exaggerated lithotomy position, the anterior vaginal wall is dissected free over the site of the diverticulum, as in repair of cysto-urethrocele, preferably through a longitudinal incision. The sac is mobilized and opened to expose the urethral communication. A catheter is placed in the urethra as a guide to dissection. Following excision of the sac close to the urethra, the urethral defect is closed transversely with interrupted sutures of No. 00 chromic catgut. Small-mouthed diverticula may also be treated by placing a purse-string suture about the stump, followed by inversion of the stump into the urethra. Plastic repair of the urethrovaginal septum may be necessary in large diverticula but in most cases simple excision of the redundant vagina mucosa and closure of the wound without drainage will suffice.

To show how frequently this condition is overlooked Bergman[2] reports from his experience 20 cases in 13 of which previous treatment had been unsatisfactory for conditions diagnosed as cystitis, urethritis, pyelonephritis, ureteral stricture, ureteritis, endocrine imbalance, colitis, arthritis, periappendicitis, salpingitis, and psychoneurosis. Treatment for relief varied from a few months to a period of 25 years in one instance. Two patients had received sulfa therapy for over a year. In most instances the removal of the sac promptly relieved symptoms.

Lymphopathia Venereum. Presenting 11 cases of lymphopathia venereum of the female urethra, which were observed in the Gynecological Department of Johns Hopkins Hospital, Gray[7] believes he has been able to indicate stages of a syndrome occurring in women, beginning with a chronic urethritis and progressing to urethral stricture or ulceration. Chronic inguinal adenitis often progressing to suppuration with multiple draining sinuses has

been described under many names. It is sometimes called "lymphogranuloma inguinale" because its microscopic picture was thought to resemble Hodgkin's disease. Unfortunately, this confusing term has since then been widely used, although it has been clearly demonstrated that there is no relation or real similarity to Hodgkin's disease. While the disease was studied by many investigators, no great progress was made until Frei, in 1925, discovered a specific diagnostic antigen prepared from the bacteriologically sterile pus from these cases. His intradermal test gave impetus to world-wide studies. By means of the Frei test several other interesting clinical conditions have been linked with so-called lymphogranuloma inguinale. The association of large labia with ulceration of the skin and vaginal mucous membrane, hypertrophic tags about the rectum, and sometimes with rectal stricture, has been recognized for very many years. The terms elephantiasis vulvae, esthiomene, chronic hypertrophic vulvitis, and genito-anorectal syndrome have been used to indicate this disease. Though the disease has no known relation to Hodgkin's disease, sometimes called "lymphogranuloma", it is often confused with it. It is also confused with granuloma inguinale, a quite different disease, primarily affecting the skin and caused by Donovan's organism. Lymphogranuloma inguinale, as shown by the Frei test, is by no means limited to the inguinal region. However, several authors have demonstrated that the inflammatory reaction characteristically extends along and blocks the lymphatics. Its venereal nature has been stressed by many. From Gray's observations, certain characteristics of the urethral lesions group themselves to form a syndrome. The disease apparently begins as a chronic urethritis, which may be accompanied by an intra-urethral ulcer, and may remain stationary for years, with or without treatment. Apparently, if the inflammatory reaction extends deeply into the tissues, the disease may proceed so slowly as to allow progressive formation of scar tissue, resulting in stricture of the lumen. The stricture may occur at any point, but is most commonly near the internal sphincter. In some cases the inflammation extends at a steady rate, involving most severely the urethral meatus, resulting in ulceration of the orifice. The ulceration may proceed, causing all degrees of destruction of the urethra and surrounding vaginal mucous membrane, although he has not seen complete destruction of the internal urethral sphincter or base of the bladder. These ulcerations have a firm, granular polypoid base, 0.5 to 2 cm. deep, with characteristic thickened, undulating, undermined borders. The base may be very red, extremely tender, and may bleed easily, or it may be gray, granular, and insensitive. The ulceration may be entirely replaced by the contraction of scar tissue, and a lesion may be partially or entirely halted in such a manner at any point. The ulceration may extend around the introitus and burrow deeply to each side of the rectum. There may or may not be an associated elephantiasis of the vulva. Six of the 11 cases presented in this paper have had syphilis and have been treated with arsenic and bismuth preparations.

In the treatment of lymphopathia venereum, numerous drugs have been used, including arsenic, antimony, manganese, and gold compounds. Antisyphilitic treatment has been shown to have no effect. Tartar emetic is most widely used, and while many observers have claimed good results, others have not met the same success. This drug acts as a specific in the treatment of granuloma inguinale, which probably explains the attempt to extend the use of tartar emetic to lymphopathia venereum. The confusion of these 2 diseases has been discussed.

Since the foregoing report was presented, there have been many advances in therapy by the use of antibiotics and in this disease an encouraging note is the report of Wright, Sanders, Logan, Prigot and Hill[18] who were the first to use aureomycin in 25 patients with this disease. Inasmuch as the disease is supposed to be due to a virus, it is logical to expect good results from aureomycin which has given such good results in other virus diseases. Their cases were divided into 3 groups: 1) buboes, 2) proctitis, and 3) benign rectal strictures. All patients gave a detailed history, and complete physical examinations and extensive laboratory studies were made. Eight patients with buboes were treated and showed a decided reduction in the size of the nodes at the end of 4 days of treatment. In 3 cases, smears from aspiration of the bubo revealed inclusion and elementary bodies, which had all disappeared within 1 week after treatment. Three patients with proctitis were treated and all improved, with cessation of rectal discharge, bleeding, and tenderness. After treatment, proctoscopic examination showed normal rectal mucosa. Fourteen patients with benign rectal stricture were treated. All showed clinical improvement, with decrease in rectal bleeding, discharge, and pain, and an increase in stool diameter. However, no gross pathologic change in the fibrous rectal stricture was apparent. Aureomycin suspended in isotonic saline solution occasionally produced a mild, easily rectified hypochromic anemia. No other toxic effects were noted.

In a later summarization on the use of antibiotics in various infections, Long, Chandler, Bliss, Bryer and Schoenbach[10] conclude that aureomycin should have the preference in the treatment of this disease, while second choice is given to Penicillin G. It may be interesting that they state that streptomycin is of no value in lymphopathia venereum but is the antibiotic of equal value with aureomycin in treatment of granuloma inguinale, which, as stated before, is sometimes confused with lymphopathia venereum.

Female Prostatism. The name of the late Hugh H. Young[19] has been intimately associated with the development of the surgery of the prostate gland, but it may not be generally remembered that he was also one of those who had experience with vesical neck obstructions in women. In such conditions he stated that the development of hesitation, difficulty in starting micturition, slowness of the stream, irritation, pain, hematuria and the presence of more or less complete retention of urine are quite similar to those conditions in men. On cystoscopic examination the mucosa of the urethra and adjacent portion of the trigone is inflamed, often edematous or even papillomatous. The trigone sometimes hypertrophies as a result of the obstruction. The bladder becomes trabeculated and sometimes cellular from back pressure and increased activity of the detrusors. Impairment of renal function may result from residual urine and back pressure effects on the ureteral orifices and the consequent dilatation of the ureters, pelves and thinning of the renal cortex. With the advent of infection the condition becomes aggravated and ultimately the results may be very serious. He quotes the work of Johnson, who made serial sections of female embryos of various lengths (60, 75, 150, 215 and 275 mm.) and from these prepared wax reconstructions that showed glands along the urethra from the vesical orifice to the meatus which corresponded to the prostatic glands in the male. These prostatic glands in the female correspond in number and give rise to branches, but they never attain the development of the male prostatic glands except when, as a result of

hyperplasia of the adrenal cortex (the androgenic zone), these prostates in females continue their growth and ultimately almost attain the development of the male prostate. These cases present a common and often very troublesome condition in women that is often overlooked. The fact that the urine is usually normal, the cultures grow no bacteria and the ordinary cystoscope shows a vesical orifice that appears normal with the right angle telescope has led many observers to pronounce the urethra normal and to seek to relieve these patients by ureteral instrumentation. This is particularly true when pain in the back with attacks of colic in the region of one or both kidneys strongly suggests ureteral abnormality. Benefit following cystoscopy and ureteral dilation not infrequently has been accepted as confirming the diagnosis, but such benefit may indeed be the result of urethral instrumentation rather than ureteral. The symptoms are apparently localized quite a distance from the urethra. It is therefore highly essential that, even when the history of the patient seems to point strongly to disease of the upper urinary tract, a very careful study of the urethra should be made, and if evidence of chronic inflammation and the various pathologic conditions described are present, local treatment for the urethra and vesical neck is indicated, certainly as a preliminary measure before the patient is subjected to a series of ureteral dilations or operations on the kidney itself. The conditions which produce such severe local and referred symptoms are readily cured by endo-urethral treatment: applications of silver nitrate, fulguration, the punch operation or excision of the obstructing bar, or severely inflamed regions either with the simple punch instrument or the electrical resectoscope.

Discussing their experience in the treatment of this condition, Folsom and O'Brien[6b] state that these lesions lend themselves very readily to transurethral resection. The results are as satisfactory as they are in the male. We should therefore recognize this as a gross clinical entity and begin to offer to these patients the great relief we have for years been affording the male who is similarly affected. In transurethral resection in the female it must be realized that we have to be much more careful in our technique: first, for the simple reason that, as we are dealing with miniature prostates we must be more sparing in our bites of tissue removed to insure against cutting through the urethrovaginal septum; second, because there is, as far as has been observed, no capsule that may be seen during resection and hence act as a guide; third, because excessive fulguration may be as bad as too deep a cut. This accident happened to these authors in 1 of their cases and on the 9th postoperative day led to the formation of a urethrovaginal fistula, which was later repaired. They therefore use as little fulguration as possible, relying on the Foley bag and traction to control the bleeding.

The condition which Powell and Powell[13] call the "urethral syndrome" is variable in its manifestations and frequently flares up following pregnancy, a surgical procedure, or an illness which confines the patient to bed. Bladder discomfort affected over 95% of the 603 patients studied and varied in severity. Referred pain was an important factor in 60% of the cases and usually was dull and referred to various regions near the pubis. Urgency of urination occurred in 28% of the cases and occasionally was severe enough to produce incontinence while frequency of urination was complained of by over 36% of the patients. In studying these patients, the causative fac-

tors for these symptoms were usually polyps, granulations, strictures or enfolding collarette of the posterior urethra. The symptoms usually responded to urethral soundings, instillations, fulgurations or occasionally resections of the posterior urethra and bladder neck.

URETHRAL INCONTINENCE. Since urinary incontinence in the female is usually due to either a faulty bladder sphincter or a fistula, it is not surprising that another cause may be overlooked. Such is often the case when an ectopic ureter empties into the urethra below the sphincter. Reviewing 19 such cases which were observed at the Mayo Clinic, Cristol and Greene[5] state that this condition presents a characteristic picture. Most frequently the patients are young women, unmarried and rather retiring. Most often they have interrupted their elementary education and those who have sought jobs usually have not remained employed in one location for any length of time. Many have had few social contacts with members of the opposite sex. Of the few who have married, several have been divorced. For as long as they can remember, they have found it necessary to protect their underclothes from a slow but constant seepage of urine. This annoyance has been present despite the fact that they have a normal desire to urinate and perform that function in a normal manner. These persons have an anomalous condition of the urinary tract which fortunately lends itself readily to operation and cure. They usually have duplication of the renal pelvis and ureter on one or both sides. The ureter from the upper renal segment empties distal to the external urinary sphincter in the urethra or vestibule of the vagina rather than in the bladder. Thus the duplication is unilateral, while the bladder normally fills with urine which comes from the lower segment of the kidney in which the duplication occurs together with the urine coming from the kidney on the opposite side, and the patient gets a normal desire to void, still urine constantly seeps from the ectopic ureteral orifice. The diagnosis almost always can be made from the history alone. Any girl or woman who has dribbled urine constantly for as long as she can remember, despite the fact that she normally has the desire to void and does urinate, probably has an ectopic ureteral orifice or orifices. Further diagnostic evidence is obtained from the excretory urogram, in which duplication of the renal pelvis and ureter may be obvious or suggested. The treatment is surgical and usually consists in heminephrectomy of the upper renal segment and partial ureterectomy. In the cases in which bilateral duplication is present and in which 2 aberrant ureteral orifices consequently may be present, the upper segments of both kidneys are removed. In such a case after the first heminephrectomy the leakage of urine is reduced but nevertheless persists until the other heminephrectomy is performed.

REFERENCES

(1) **Auer, E. S.**: Am. J. Obst. and Gyn., **30**, 318, 1935.

(2) **Bergman, R. T.**: Urol. and Cut. Rev., **53**, 590, 1949. (3) **Berry, N. E.**, and **Greene, H.**: J. Urol., **39**, 92, 1938.

(4) **Counseller, V. S.**: Am. J. Obst. and Gyn., **57**, 231, 1949. (5) **Cristol, D. S.**, and **Greene, L. F.**: J. Am. Med. Assn., **130**, 1061, 1946.

(6a) **Folsom, A. J.**, and **O'Brien, H. A.**: Ibid., **128**, 408, 1945. (6b) Idem: Ibid., **121**, 573, 1943.

(7) **Gray, L. A.**: Surg., Gyn. and Obst., **62**, 745, 1936.

(8) **Hess, E.:** Penna. Med. J., **48**, 1150, 1945. (9) **Huffman, J. W.:** Am. J. Obst. and Gyn., **55**, 86, 1948.

(10) **Long, P. H., Chandler, C. A., Bliss, E. A., Bryer, M. S.,** and **Schoenbach, E. B.:** J. Am. Med. Assn., **141**, 315, 1949.

(11) **Palmer, J. K., Emmett, J. L.,** and **McDonald, J. R.:** Surg. Gyn. and Obst., **87**, 611, 1948. (12) **Pomeroy, L. A.:** Am. J. Roentgenol., **35**, 259, 1936. (13) **Powell, N. B.,** and **Powell, E. B.:** J. Urol., **61**, **557**, **1949.**

(14) **Stevens, W. E.:** J. Am. Med. Assn., **106**, 89, 1936.

(15) **Trafton, H. M.:** Surg. Clin. N. Am., June, p. 589, 1945.

(16) **Walther, H. W. E.:** J. Urol., **50**, 380, 1943. (17) **Watson, E. M.:** Ibid., **35**, 654, 1936. (18) **Wright, L. T., Sanders, M., Logan, M. A., Prigot, A.,** and **Hill, L. M.:** J. Am. Med. Assn., **138**, 408, 1948.

(19) **Young, H. H.:** Ibid., **115**, 2133, 1940.

BINDINGS FOR COMPLETED VOLUMES

The desire to preserve the issues of THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES for permanent reference has brought numerous requests from subscribers for information as to how they may have their copies bound.

We are pleased to announce that The Book Shop Bindery, 308 West Randolph Street, Chicago 6, Illinois, has been selected as offering the finest service. They will bind the 6 issues of the Volume in the best grade of washable brown buckram with gold stamping on the spine and the subscriber's name in gold on the front cover. A check for $3.15 payable to the Book Shop Bindery should accompany order. Bound volumes will be returned prepaid.

UNDER THE CHARGE OF

IRVING J. WOLMAN, M.D.

ASSISTANT PROFESSOR OF PEDIATRICS, UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA, PENNSYLVANIA

EVALUATION OF ADRENAL CORTICAL FUNCTION IN PEDIATRICS: METHODS

BY ALFRED M. BONGIOVANNI, M.D.
PHILADELPHIA, PENNSYLVANIA

(From the Children's Hospital of Philadelphia, Department of Pediatrics, University of Pennsylvania, School of Medicine)

AN understanding of the presently available methods for estimating adrenal cortical function in children is necessitated by a greater awareness among medical men of the importance of the adrenal cortex in clinical pediatric study. This has arisen from a fuller comprehension of endocrinologic disorders in general and of certain primary adrenal disturbances that may be of unique pediatric concern (such as female pseudohermaphroditism with clinical manifestations of adrenal insufficiency[39]). Furthermore the broad application of Selye's[63] "general adaptation" syndrome warrants investigation of the adrenal cortex in a variety of pediatric diseases. Finally, there has been the recent availability of hormonal substances (adrenocorticotropin and cortisone) for therapeutic and investigative use, which have shown promise in numerous conditions of childhood; their intelligent clinical use requires a knowledge of some pertinent laboratory methods.

Methods for estimating adrenal cortical function depend in large part on the excretion of metabolites and renal overflow of cortical hormones, which may be measured in the urine in various ways. These metabolites represent mixtures of steroids, many of which remain unidentified to date. Only those procedures that are relatively simple and practicable in clinical study will be described. More refined techniques, such as those using a chromatographic column[13], allow almost complete separation of the various steroids and enhance their identification. However these remain the tools of research for the present. It must be understood that these methods are in the process of evolution and may be discarded for more desirable ones within the next few years.

CHEMICAL CONSIDERATIONS. It is necessary briefly to review the structure and properties of adrenal steroids. The steroid nucleus is shown in Fig. 1. Most adrenal steroids contain 19 or 21 carbon atoms, 2 of which are angularly located at C 10 and C 13. Those with only 19 carbon atoms and a ketonic group at C 17 as typified by androsterone (II) in the figure, are designated 17-ketosteroids. Physiologically such hormones may be anabolic or androgenic and arise as excretory products from the adrenal cortex, but in part from the testes in the male. In Addi-

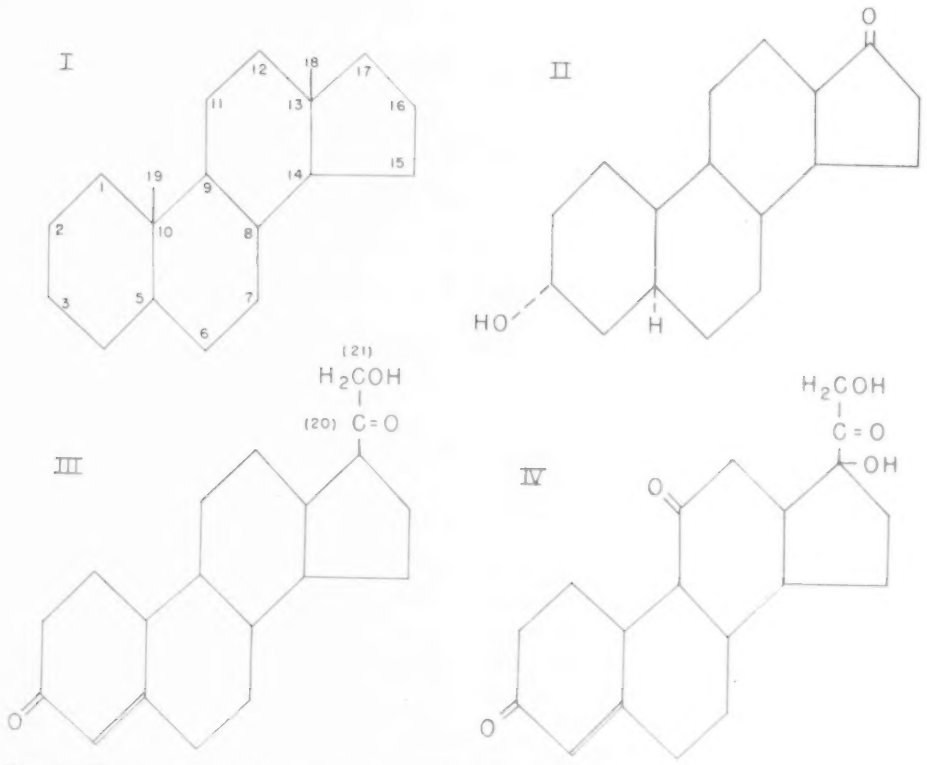


FIG. 1.—The structure of adrenal cortical hormones. I. The steroid nucleus. II. Androsterone (androstan-3-a-ol-17-one). III. Desoxycorticosterone (Δ^4-pregnen-21-ol-3, 20-dione). IV. Kendall's Compound E, cortisone (17-hydroxy-11 dehydrocorticosterone).

TABLE 1.—STEROID EXCRETION IN CHILDREN

Age	*Ranges of Averages (mg. per 24 hours)*			
	Chemical			
	17-ketosteroids	*Reducing*	*Formaldehydogenic*[39]	*Biologic (glycogenic)*[35,72,73,74]
Premature		0.17–0.38[66]*		
Newborn	0.13–0.67[10]	0.26–0.62[66]*	0.09 –0.26	0–0.017
	0.5 –1.5[54]	0.087[10]	0.025–0.133[54]	
1 year			0.22 –0.26	
2–6 years	0.15–0.5[66]	0.32–0.70[66]*	0.23 –0.31	0.034–0.053
7–9 years	0.65–1.4[66]	0.79[66]*	0.22 –0.26	0.058
10–14 years	1.9 –5.3[66]		0.29 –0.36	
15–18 years	6.3 –8.1[66]		0.45	
Adult Male	11.0[66]	1.1 –2.1[66]*	0.52	0.062
Female	6.8[66]	1.0 –2.0	0.60	0.039

* phosphomolydate reducing

son's disease their urinary titer is almost zero in the female, but about 30% of normal in the male[27]. Because of the presence of the 17-ketone group these compounds are characterized by certain color reactions which are taken advantage of in methods for their determination. The hydroxyl group at C 3 may be oriented above or below the plane of the molecule. These are designated as β (solid bond) or α (broken bond) respectively. In normal individuals the 3 (α)-hydroxyl compounds constitute the major portion of the urinary 17-ketosteroids. It is possible chemically to separate and measure the α and β compounds. The value of such a separation will be discussed below.

The C 21 steroids possess a 2 carbon atom side chain at C 17. These may be hydroxylated at C 17 as in compound E or the fourth valence may be satisfied with hydrogen. The former are easily converted into 17-ketosteroids by mild oxidation and may therefore represent metabolic precursors of some C 19 steroids measured in urine. In all compounds C 11 is either oxygenated (ketonic or alcoholic) or unsubstituted. The unsubstituted steroids alone show marked action as regards salt and water metabolism (desoxycorticosterone.) The C 11 and C 11-17 oxygenated series have high gluconeogenic potency (compound E of Kendall) and little or no mineral and water activity. In both groups the presence of a ketonic oxygen in the side chain at C 20, adjacent to a primary hydroxyl group at C 21, is essential to cortical activity and imparts reducing properties to the molecule. In addition, oxidation of the compounds with the described side chain produces formaldehyde. These chemical properties (reduction and formaldehyde production) are the bases of the methods to be considered.

Other sources may be consulted for further details of the structure, chemistry, nomenclature and properties of adrenal cortical hormones[23,31,40,45,57].

Methods. For convenience the methods of evaluating adrenal cortical activity will be divided into clinical, chemical, and biological.

Classical clinical procedures that have been used in the study of adrenal disease will not be detailed. These consist of glucose and insulin tolerance, salt deprivation[8,30,77], water[58], and potassium tolerance tests[8,79].

Dougherty and White[17] have noted a relationship between circulating lymphocytes and the adrenal cortex. Lymphocytopenia results from increased adrenal cortical activity produced by stress or ACTH. This response is not forthcoming in the adrenalectomized animal. However, the role of the eosinophils in this same regard, as observed by Hills[35], offers a more practical method for evaluating adrenal cortical function. The eosinopenia following adrenocortical stimulation is more sensitive and less variable than the lymphocytopenia.

Forsham[25] and Thorn *et al.*[69] have described a simple method for determining the integrity of the adrenal cortex following a single dose of ACTH. In the presence of normal cortical reserve there follows a diminution in circulating eosinophils (50 to 80%) in 4 hours. The normal initial eosinophil levels vary widely between 80 and 500 eosinophils per c.mm. of blood; these are higher in infants, numbering between 468 and 969 according to Read *et al*[54]. The circulating eosinophils are counted in oxalated venous blood using a white cell diluting pipette and the usual counting chamber, although one which is 0.2 mm. deep is recommended. The diluting fluid recommended by Thorn[69] is that of Dunger[19]:

eosin (2% aqueous)	5.0 cc.
acetone	5.0
water q.s.	100.0

This solution stains eosinophils deeply and facilitates counting. However it has

the disadvantage of readily causing disruption of eosinophils and the count must be completed shortly following dilution. Another solution employing phloxine in propylene glycol[34,52a,b,53] is believed to be more accurate and permits the count to be completed in a more leisurely manner since the eosinophils remain preserved for a long period of time. A suitable preparation is as follows[34]:

Phloxine, 0.05 gm., propylene glycol, 50 cc., water, 50 cc.

In addition to the alterations in eosinophil counts, a rise in the urinary uric acid/creatinine ratio constitutes a normal response to ACTH[69]. However the latter change is not a satisfactory index in infants[68].

The eosinophil response alone is not always a reliable indication of adrenal cortical activation[68]. The eosinophil count may fall without a significant rise in urinary steroid levels or a clinical response. Conversely, in allergic eosinophilia the count may not fall in spite of increased urinary steroid excretion and a clinical response. In addition, 2 types of eosinophil responses have been noted[42] under some circumstances (following histamine injection): immediate and delayed. There is evidence that the immediate fall after histamine may not be mediated via the release of adrenal hormones. Precise clinical investigation with ACTH should not rest upon the eosinophil response alone.

The above test may also be conducted by using subcutaneous epinephrine (0.3 mg.) instead of ACTH[69]. It probably acts by stimulation of the hypophysis and secondary stimulation of the adrenal[56]. However Recant *et al.*[55] noted eosinopenia with 1.5 mg. epinephrine intravenously in patients with Addison's disease which suggests that epinephrine has a direct effect on the eosinophils.

The eosinopenic response appears to depend upon C 11,17 oxygenated steroids as typified by compond E. Desoxycorticosterone does not produce a fall in the eosinophil count. Hence the observed changes in the eosinophils presumably measure the production of the former type of steroid.

A clinical measure of the mineralcorticoids (11-desoxy) controlling salt and water metabolism is available on the basis of Conn's[6] investigations. The sodium concentration of sweat appears to be a sensitive and reliable index to desoxycorticosterone-type activity. It is sensitive to the administration of exogenous desoxycorticosterone and may be used to assess endogenous production of related substances. The collection of sweat has been described by Conn in adults, but is cumbersome and often impossible in children. Far more simple and subject to the same influences is the concentration of sodium in saliva. Eisenmenger *et al.*[21] and the author[3] have applied the latter to the study of adrenal cortical activity with satisfaction. Sufficient figures are not available to define the normal pediatric limits at present.

Bioassay. Methods of bioassay of urinary extracts depend upon the physiological properties of the steroids present. The extracts are injected into adrenalectomized animals and a particular physiologic effect is observed in terms of a reference standard, usually measured quantities of a known pure steroid. The methods depend upon growth and survival, work performance, hepatic glycogen deposition, urinary deposition, urinary sodium retention, resistance to cold exposure or other stress, prevention of water and potassium intoxication. These are briefly reviewed and evaluated by Dorfman[14].

The bioassay is somewhat complicated and perhaps too involved for routine clinical purposes. However, it yields specific information in terms of physiologic significance that cannot be

obtained by the simpler chemical techniques alone. And yet it is more simple than precise chromatography such as has resulted in the isolation of cortisone from normal urine[62].

Two techniques which have been helpful in clinical investigation may be described. 1. Following adrenalectomy, fasting mice show an almost complete loss of liver glycogen. The deposition of liver glycogen may then be induced by C 11-17 oxysteroids (compound E), in quantity proportional to the dose of the steroid. This is the basis of a method[16,20,73] which may be applied to clinical study. It represents the level of urinary steroids possessing this particular characteristic only, and may vary from normal in spite of the presence of usual total levels measured chemically.

2. Deming and Luetscher[11] have recommended the use of adrenalectomized rats for the measurement of "salt-water" active steroids in urine, the level being related to the degree of sodium retention produced in a fixed period of time. This is sensitive to about 10 gamma of desoxycorticosterone. While apparently not possessing, in our hands, the degree of accuracy claimed for it, it has been useful in clinical study[3]. Dorfmann's[14] modification recommends the use of radioactive sodium and is far more sensitive.

Chemical. The 17-ketosteroids, as measured routinely, represent a heterogenous group of steroids with a ketonic oxygen at C 17. They probably include C 19 hormones of the adrenal cortex as such (and the testes) and the metabolic products of some C 21 steroids. These are excreted in the urine as water soluble conjugates. Their extraction involves hydrolysis of the conjugates, and their removal with organic solvents in separatory funnels or continuous extractors. The extracts are washed with a base which removes phenolic steroids (estrogens), chromogenic material and acids. The quantity of 17-ketosteroids is then determined colorimetrically by the Zimmerman reaction with m-dinitrobenzene in alkaline solution, or the Pincus[50] reaction with antimony trichloride in acetic acid. The former method has been used most, following the fundamental specifications of Callow *et al.*[5]

Various simplifications of the methods utilizing the m-dinitrobenzene reaction have been described[2,18,58] which require smaller quantities of urine and can be performed more rapidly. That of Drekter *et al.*[18], with further modifications for children, has been satisfactory in our hands.

The α and β stereo-isomers of 17-ketosteroids have been described. These may be separated by the precipitation of the β compounds with digitonin. A useful micromethod has been reported[26]. Normally the β fraction is less than 20% of the total. This fractionation is a valuable aid in differentiating adrenal tumor from adrenal hyperplasia[38]. In both, the excretion of 17-ketosteroids is usually elevated; however in tumor the β fraction is often as high as 50% or more of the total. There are limitations to the value of this fractionation and the relationships are not invariably as presented[38].

Dehydroiso-androsterone is the principal component of the β fraction of urinary steroids in adrenal cortical tumor. Relatively simple methods utilizing a color reaction to measure this substance in urine extracts have been described[1,49]. A direct estimation of this steroid may be helpful and is simpler than fractionation.

Corticoids. The urinary "corticoids" designate compounds other than 17-ketosteroids. The usual chemical means for their determination depend upon properties characteristic of C 21 cortical hormones but which are not individually identified. These methods are not entirely specific for cortical compounds and the reactions involved

may be influenced by other unrelated organic substances. Determinations are conducted on crude urinary extracts following relatively mild hydrolysis.

It has been indicated that C 21 steroids with the appropriate 2 carbon side chain at C 17 (desoxycorticosterone and 17 hydroxy 11 dehydro corticosterone) have reducing properties and on mild oxidation release formaldehyde.

The reduction of phosphomolybdic acid[32,33] or copper[65] by the lipid soluble residue of urine forms the basis for 2 systems of estimating urinary "corticoids". Phosphomolybdic acid is reduced both by the α ketol function of the side chain at C 17 and the α,β unsaturated 3-ketone group. Copper is apparently reduced only by the former.

The generation of formaldehyde by the oxidation of steroids of the type described underlies the method first described by Lowenstein, Corcoran and Page[43]. The formaldehyde released is distilled from the oxidized urine residue and measured in the distillate by chromogenic agents[7,9].

Normal values differ considerably according to the method used. In the experience of the writer the phosphomolybdate reduction of Heard and Sobel[32,33], while perhaps somewhat less specific, is more practicable and quite reliable for routine purposes. Although the absolute values are different from those of the formaldehydogenic methods, its reliability is attested to by Romanoff *et al.*[61]. Variations in titer according to one method, are paralleled in the other, in spite of the fact that absolute figures differ.

Various refinements of these methods are available, but these add to the burden of procedures which are already involved and for the present the above systems are satisfactory. Finally, the independent significance of the 17-ketosteroids and "corticoids" must be pointed out. The lack of parallelism between the excretion of 17-ketosteroids and "corticoids" has been interpreted as evidence of a dual role of the adrenal cortex. That this duality does in fact become prominent in certain diseases will be shown below.

Normal Pediatric Values. The normal values for the urinary excretion of the steroids discussed are tabulated from several sources for the pediatric age groups. In all instances the 17-ketosteroids were determined by the Zimmerman dinitrobenzene reaction. The absolute values for "corticoids" differ according to the method. In most instances the studies are based on males, and there is insufficient data to establish a significant difference between sexes in the younger age groups.

According to Read *et al.*[54], the excretion of 17-ketosteroids generally exceeds 1.5 mg. per day in the first few days of life, falling thereafter below 0.5 mg. Day[10] reports values as high as 3.37 mg. daily in newborn infants, but the unusually large quantities were excreted by infants born via Caesarian section. Talbot *et al.*[66] have shown that under 8 years of age the 17-ketosteroid excretion is very low (less than 1.0 mg.). A rapid rise occurs between 8 and 12 years and by the 12th birthday at least 1.0 mg. per day is expected.

King *et al.*[39] performed 121 determinations of "corticoids" on 95 children using formaldehydogenic techniques. They report a rise of 0.01 mg. per year of age. In this study children between 3 and 13 years responded to stress by a significant rise in corticoids. Read *et al.*[54,70] showed that the urinary excretion of formaldehydogenic steroids varied between 0.025 and 0.133 mg. per day in a neonatal group, which was comparable to adult values on the basis of surface area. Day[10], and Matson and Longwell[46] indicated that the excretion of reducing steroids in the newborn

was also comparable to adult levels.

Pincus *et al.*[51] have shown diurnal variations in the excretion of 17-ketosteroids and reducing corticoids. A 24-hour urine specimen should be used whenever possible for this reason.

Read *et al.*[54] report that the normal eosinophil count in the neonatal period is high: 468 to 969 per c.mm. They have also shown that eosinopenia follows the administration of ACTH to infants, which is more marked with repeated doses. The urinary "corticoids" rose after the administration of ACTH, more markedly on the 9th than on the 2nd day of life. Single small doses produced no rise. The 17-ketosteroids were also shown to increase in infancy after the administration of this hormone.

Disease. The deviations in the excretion of steroids in disease will be discussed to a limited extent in order to apply the above presentation to clinical problems. This is not intended as a complete catalogue of adrenal cortical disease, primary or secondary.

Changes in the excretion of steroids may be due to: 1, primary adrenal disease (hyper- or hypoadrenalism): 2, variations in adrenal function secondary to other disease in accordance with Selye's[6] concepts of stress and exhaustion, or secondary to disease of other endocrine glands; 3, the abnormal metabolism and excretion of steroids in various diseases in which adrenal cortical function itself may be little changed. These conditions may be reflected in rises and falls in the excretion rates of 17-ketosteroids or corticoids (which may vary independently); by alterations in the eosinophil count, salt tolerance, saliva and sweat sodium concentrations.

Increased steroid excretion generally indicates hyperactivity of the adrenal cortex. This occurs in Cushing's disease whether due to pituitary basophilism, adrenal hyperplasia or adrenal cortical tumor. Cushing's syndrome occurs in children as such[22]. When Cushing's syndrome is accompanied by increases of 17-ketosteroid in the urine, fractionation into α and β stereoisomers may aid in the differentiation of tumor from hyperplasia. In 6 patients with tumors of the adrenal cortex, Kepler *et al.*[38] report a high β fraction in 4. Mason[44] reviews 16 instances of adrenal cortical tumor with distinct elevations of the β fraction in 12 amounting to 30 to 87% of the total. However he emphasized that neoplasm may be present in spite of a normal β fraction and that the total quantity of 17-ketosteroids may be absolutely greater in hyperplasia.

Elevation of chemical urinary "corticoids" has been reported by Bongiovanni and Eisenmenger[3] in chronic hepatic disease (Laennec's cirrhosis) with ascites. It is possible in this condition, that the "corticoids" are not normally disposed of by the liver and hence overflow, rather than indicating actual adrenal cortical hyperactivity.

Salt and water retaining hormones have been demonstrated to occur in large quantities, by bioassay, in the urine of patients with nephrosis and heart failure[11]. Parrish[48] has described increased corticoids of life prolonging type in heart failure. Similar results have been reported by others.

Rises in infancy have occurred following stress[39], and normally after the administration of ACTH[54,70]. Hence one may expect a rise early in the course of acute illness or injury, although prolongation of the stress may result in exhaustion. Failure to respond to ACTH may be interpreted as due to primary adrenal insufficiency, should there be no rise in steroid excretion or fall in eosinophil count[25,69]. It has been shown that the elevation in uric acid excretion is of no value in infancy[54]. The response to epinephrine is included above and depends upon the

integrity of the hypophysis as well as the adrenal[55]. Roche *et al.* [60] have utilized such responses to ACTH as a means of predicting the ability of patients to withstand surgery. Where adrenal cortical reserve appears adequate, it may be assumed that the stress of surgery will be well tolerated.

Specific steroids may occur in the urine in some conditions as measured by chromatographic techniques. Most interesting has been the discovery of 11-hydroxyetiocholanolone in the urine of many patients with malignant tumors (mammary and gastric)[12]. This is beyond the scope of ordinary clinical laboratory tests, however.

Adrenal insufficiency as in classical Addison's disease will be accompanied by a low excretion of the various steroids, chemically and biologically. In early childhood, because of the very small quantities of steroids in urine, it may be difficult to use such methods for evaluation. The responses to ACTH or epinephrine can be more helpful.

Diminution in steroid excretion may be secondary to chronic debilitating diseases of non-specific etiology[27], starvation[41], primary pituitary insufficiency and retarded growth in older children[66]. In hepatic cirrhosis and acute hepatitis the 17-ketosteroids are low in titer, but recent studies[3] suggest that this may be due to a failure of the hepatic glucuronide conjugation of steroids so that they may not be excreted in the urine as water soluble compounds.

The independent variation of 17-ketosteroids and "corticoids" has been shown to occur in cirrhosis[3]. It is best illustrated in the adrenogenital syndrome, wherein even the clinical features suggest hypoactivity of some adrenal functions and hyperactivity of others[4,36a,b,47,67,76,78]. There is evidence of hypoadrenia in the lack of salt and water control with the shock like picture of Addisonian crisis. This is associated with macrogenitosomia in males and pseudohermaphroditism at birth, in females. The excretion of 17-ketosteroids (androgenic) is high, even in infants, and the "corticoids" are expected to be low. However, the latter may be normal[4].

Finally, it should be realized that adrenal cortical disease has been associated with the increased excretion of other hormones than those discussed, (estrogenic)[75]; or that elevated urinary steroids may originate from sources other than the adrenal, as in testicular tumors with high 17-ketosteroid excretion.

Conclusion. A few of the more practical methods for estimating adrenal cortical function in the pediatric age group have been described. There are many others, and newer, simpler techniques are being evolved. Deviations in adrenal cortical hormone metabolism in pediatrics have been illustrated in a representative group of diseases. Further studies along these lines, in children, are indicated.

REFERENCES

(1) **Allen, W. M., Hayward, S. J.,** and **Pinto, A.:** J. Clin. Endocrinol., **10,** 54, 1950.

(2) **Bjorneboe, M., Hamburger, C.,** and **Jerseed, M.:** Acta med. Scandinav., **136,** 287, 1950. (3) **Bongiovanni, A. M.,** and **Eisenmenger, W. J.:** J. Clin. Endocrinol., in press. (4) **Butler, A. M., Ross, R. A.,** and **Talbot, N. B.:** J. Pediat., **15,** 831, 1939.

(5) **Callow, N. H., Callow, R. K.,** and **Emmens, C. W.:** Biochem. J., **32,** 1312, 1938. (6) **Conn., J. W.,** and **Louis, L. H.:** J. Clin. Endocrinol., **10,** 12, 1950. (7) **Corcoran, A. C.,** and **Page, I. H.:** J. Lab. and Clin. Med., **33,** 1326, 1948. (8) **Cutler, H. H., Power, M. H.,** and **Wilder, R. M.:** J. Am. Med. Assn., **111,** 117, 1938.

(9) **Daughaday, W. H., Jaffe, H.,** and **Williams, R. H.:** J. Clin. Endocrinol., **8,** 166, 1948.

(10) **Day, E. M. A.**: Med. J. Australia, **35**, 122, 1948. (11) **Deming, Q. B.**, and **Luetscher, J. A.**: Proc. Soc. Exp. Biol. and Med., **73**, 171, 1950. (12) **Dobriner, K., Lieberman, S., Hauton, L., Sarrett, L. H.**, and **Rhoads, C. P.**: J. Biol. Chem., **169**, 221, 1947. (13) **Dobriner, K., Lieberman, S.**, and **Rhoads, C. P.**: Ibid., **172**, 241, 1948. (14) **Dorfman, R. I.**: Ann. N. Y. Acad. Sci., **50**, 556, 1949. (15) **Dorfman, R. I., Potts, A. M.**, and **Feil, M. L.**: Endocrinology, **41**, 461, 1947. (16) **Dorfman, I., Ross, E.**, and **Shipley, R. N.**: Ibid., **38**, 178, 1946. (17) **Dougherty, T. F.**, and **White, A.**: J. Lab. and Clin. Med., **32**, 584, 1947. (18) **Drekter, I. J., Pearson, S., Bartazak, E.**, and **McGavack, T. H.**: J. Clin. Endocrinol., **7**, 795, 1947. (19) **Dunger, R.**: Munchen med. Wchnschr., **57**, 1942, 1910.

(20) **Eggleston, N. M., Johnston, B. J.**, and **Dobriner, K.**: Endocrinology, **38**, 197, 1946. (21) **Eisenmenger, W. J., Blondheim, S. H., Bongiovanni, A. M.**, and **Kunkel, H. G.**: J. Clin. Invest. In press.

(22) **Farber, J. E., Gustina, F. J.**, and **Postoloff, A. V.**: Am. J. Dis. Child., **65**, 593, 1943. (23) **Fieser, L. E.**, and **Fieser, M.**: Natural Products Related to Phenanthrene, 3d ed., New York, Rheinhold, 1949. (25) **Forbes, A. P., Donaldson, E. C., Reifenstein, E. C.**, and **Albright, F.**: J. Clin. Endocrinol., **7**, 264, 1947. (25a) **Forsham, P. H., Thorn, G. W., Prunty, F. T. G.**, and **Hills, A. G.**: Ibid., **8**, 15, 1948. (26) **Frame, E. G.**: Endocrinology, **34**, 175, 1944. (27) **Fraser, R. W., Forbes, A. P., Albright, F., Sulkowitch, H.**, and **Reifenstein, E. C.**: J. Clin. Endocrinol., **1**, 234, 1941.

(28) **Gilder, H.**, and **Hoagland, C. L.**: Proc. Soc. Exp. Biol. and Med., **61**, 62, 1946.

(29) **Hamburger, C.**: Acta Endocrinol., **1**, 375, 1948. (30) **Harrop, G. A., Weinstein, A., Soffer, L. J.**, and **Trescher, J. H.**: J. Am. Med. Assn., **100**, 1850, 1933. (31) **Heard, R. D. H.**: The Hormones, p. 549, New York, Academic Press, 1948. (32) **Heard, R. D. H.**, and **Sobel, H.**: J. Biol. Chem., **165**, 687, 1946. (33) **Heard, R. D. H., Sobel, H.**, and **Venning, E. H.**: Ibid., **165**, 699, 1946. (34) **Henneman, P. H., Wexler, H.**, and **Westenhaven, M. H.**: J. Lab. and Clin. Med., **34**, 1017, 1949. (35) **Hills, A. G., Forsham, P. H.**, and **Finch, C. A.**: Blood, **3**, 755, 1948.

(36) **Jaudon, J. C.**: (a) J. Pediat., **29**, 696, 1946; (b) Ibid., **28**, 737, 1946.

(37) **Kenigsberg, S., Pearson, S.**, and **McGavack, T. H.**: J. Clin. Endocrinol., **9**, 426, 1950. (38) **Kepler, E. J.**, and **Mason, H. L.**: Ibid., **7**, 543, 1947. (39) **King, N. B.**, and **Mason, H. L.**: Ibid., **10**, 479, 1950. (40) **Kuizenga, M. H.**: in **Moulton, F. R.**, The Chemistry and Physiology of Hormones, p. 57, Washington, Science Press, 1944.

(41) **Landau, B. L., Knowlton, K., Anderson, D., Brandt, M. B.**, and **Kenyon, A. T.**: Endocrinology, **8**, 133, 1948. (42) **Last, J. H. Jordan, P., Petesky, I.**, and **Bond, E.**: Science, **112**, 47, 1950. (43) **Lowenstein, B. E., Corcoran, A. C.**, and **Page, I. H.**: Endocrinology, **39**, 82, 1946.

(44) **Mason, H. L.**, and **Engstrom, W. W.**: Physiol. Rev., **30**, 321, 1950. (45) **Mason, H. L.**, and **Fieser, M.**: J. Clin. Endocrinol., **8**, 190, 1948. (46) **Matson, C. F.**, and **Longwell, B. B.**: Ibid., **9**, 646, 1949.

(47) **Panos, T. C.**: Pediatrics, **5**, 972, 1950. (48) **Parrish, A. E.**: J. Clin. Invest., **28**, 45, 1949. (49) **Patterson, J.**: Lancet, **2**, 580, 1947. (50) **Pincus, G.**: Endocrinology, **32**, 176, 1943. (51) **Pincus, G., Romanoff, L. P.**, and **Carlo, J.**: J. Clin. Endocrinol., **8**, 221, 1948.

(52) **Randolph, T. G.**: (a) Proc. Soc. Exp. Biol. and Med., **52**, 20, 1943; (b) J. Allergy, **15**, 89, 1944. (53) **Randolph, T. G.**, and **Rollins, J. P.**: Proc. of First ACTH Conf., p. 1, Phila., Blakiston, 1950. (54) **Read, C. H., Venning, E. H.**, and **Ripstein, M. P.**: J. Clin. Endocrinol., **10**, 845, 1950. (55) **Recant, L., Forsham, P. H.**, and **Thorn, G. W.**: Ibid., **8**, 589, 1946. (56) **Recant, L. Hume, D. M., Forsham, P. M.**, and **Thorn, G. W.**: Ibid. In press, cited by Roche[60]. (57) **Reichstein, T.**, and **Shoppee, C. W.**: Vitamins and Hormones, **1**, 345, 1945. (58) **Robbie, W. A.**, and **Gibson, R. B.**: J. Clin. Endocrinol., **3**, 200, 1943. (59) **Robinson, F. J., Power, M. H.**, and **Kepler, E. J.**: Proc. Staff Meet. Mayo Clinic, **16**, 577, 1941. (60) **Roche, M., Thorn, G. W.**, and **Hills, A. G.**: New England J. Med., **242**, 307, 1950. (61) **Romanoff, L. P., Pflager, J.**, and **Pincus, G.**: Endocrinology, **45**, 10, 1949.

(62) **Schneider, J. J.**: Science, **111**, 61, 1950. (63) **Selye, H.**: J. Clin. Endocrinol., **6**, 117, 1946. (64) **Sniffen, R. L., Zygomintowicz, A. S.**, and **Talbot, N. B.**: Pediatrics, **5**, 808, 1950.

(65) **Talbot, N. B., Saltzman, A. H., Wixom, R. L.**, and **Wolfe, J. K.**: J. Biol. Chem., **160**, 535, 1945. (66) **Talbot, N. B., Butler, A. M., Berman, R. A., Rodriguez, P. M.**, and **MacLachlan, E. A.**: Am. J. Dis. Child., **65**, 364, 1943. (67) **Thelander, H. E.**, and **Cholffin, M.**: J. Pediat., **18**, 779, 1941. (68) **Thorn, G. W., Forsham, P. H., Frawley, T. F., Hill, S. R., Roche, M.**, and **Staehelin, D.**: New England J. Med., **242**, 783, 1950. (69) **Thorn, G. W., Forsham, P. H., Prunty, F. T. G.**, and **Hills, A. G.**: J. Am. Med. Assn., **137**, 1005, 1948.

(70) **Venning, E. H.:** Proc. of First ACTH Conf., p. 25, Phila., Blakiston, 1950. (71) **Venning, E. H.,** and **Browne, J. S. L.:** Ann. N. Y. Acad. Sci., **50,** 627, 1949. (72) **Venning, E. H.,** and **Kazmin, V.:** Endocrinology, **39,** 131, 1946. (73) **Venning, E. H., Kazmin, V. E.,** and **Bell, J. C.:** Ibid., **38,** 79, 1946. (74) **Venning, E. H., Randall, J. P.,** and **Gyorgy, P.:** Ibid., **45,** 430, 1949.

(75) **Wilkins, L.:** J. Clin. Endocrinol., **8,** 111, 1948. (76) **Wilkins, L., Fleischmann, W.,** and **Howard, J. E.:** Endocrinology, **26,** 385, 1940. (77) **Willson, D. M., Robinson, F. J., Power, M. H.,** and **Wilder, R. M.:** Arch. Int. Med., **69,** 460, 1942.

(78) **Zuelzer, W. W.,** and **Blum, A.:** J. Pediat., **35,** 344, 1949. (79) **Zwemer, R. L.,** and **Truszowski, R.:** Proc. Soc. Exp. Biol and Med., **35,** 424, 1930.

NOTICE AND INSTRUCTIONS TO CONTRIBUTORS

MANUSCRIPTS intended for publication in THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES, and correspondence, should be sent to the Editor, DR. EDWARD B. KRUMBHAAR, School of Medicine, University of Pennsylvania, Philadelphia 4, Pa. Articles are accepted for publication in THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES exclusively, except in the case of subsequent publication in Society proceedings.

MANUSCRIPTS should be typewritten on one side of the paper only, and should be double spaced with liberal margins. The author's chief position and, when possible, the Department from which the work is produced should be indicated in the subtitle. ILLUSTRATIONS accompanying articles should be numbered and have typed captions bearing corresponding numbers. For identification they should also have the author's name written on the margin or back. The recommendations of the American Medical Association Style Book should be followed. REFERENCES should be numbered and at the end of the articles arranged alphabetically according to the name of the first author and should be complete, that is, author's name, journal, volume, page and year (in Arabic numbers).

RETURN POSTAGE should accompany all manuscripts but will be returned to the author if the manuscript is accepted.

100 REPRINTS will be supplied gratis. Covers will be omitted on all articles. Orders of additional reprints will be supplied in multiples of 100.

PROCEEDINGS OF

The Physiological Society of Philadelphia

SESSION OF OCTOBER 17, 1950

Circulatory and Respiratory Reflexes from the Perfused Heart of the Dog. Domingo M. Aviado, Jr., M.D., T.H. Li, M.D., W. Kalow, M.D., G.W. Peskin, G.L. Turnbull, and M.E. Hess (Dept. of Pharmacology, School of Med., Univ. of Penna.). The demonstration that cardiac receptors are responsible for the reflex circulatory depression caused by the veratrum alkaloids (Am. J. Med. Sci., **218**, 115, 1949) necessitated this investigation of their physiological significance. The right side, the left side of the heart and great vessels of the dog (morphine and chloralose anesthesia) were each perfused independently from each of the other 2 structures and from the dog's own lungs, peripheral circulation and medullary centers. With the aid of donor dogs and Dale-Schuster pumps, the intracardiac and intravascular blood pressure and blood gas contents were changed while measuring the cardiac, vasomotor, and respiratory responses. Veratridine served as an efficient tool for testing each preparation for intact cardiac innervation and for functional ability of respiratory and circulatory systems to respond reflexly.

The existence of the following reflexes was established: 1, Increased pressure in the right side of the heart caused bradycardia, peripheral vasodilatation and slight respiratory inhibition due to receptors in the right auricle; 2, Increased pressure in the left side of the heart caused similar inhibition of respiration and circulation with receptors in the left ventricle; 3, Increased pressure in the pulmonary bifurcation usually caused a bradycardia. These 3 reflexes, which are dependent on an intact vagus, have similar effects to those caused by other presso-receptors known to exist in the carotid sinus, aortic arch and pulmonary veins (Am. J. Med. Sci., **219**, 228, 1950); 4, Tachycardia was elicited in some instances of increased pressure in the pulmonary bifurcation, but never from similar procedures in the venae cavae nor in the right auricle. The reflexes postulated to exist in the right auricle and venae cavae of intact dogs by Bainbridge, McDowall and Harrison were not confirmed; 5, Changing the blood oxygen and blood CO_2 contents in the heart and great vessels did not reflexly affect circulation; the respiratory effects are still being investigated.

Thus, there is a striking similarity in effects between the veratrum reflex and the presso-reflex in the perfused heart, though their identity remains to be proven.

The Relation of Plasma Volume and Thiocyanate Space to Total Body Water and to Body Size. James D. Hardy, M. D., and David L. Drabkin, M. D. (Harrison Dept. Surg. Research and Dept. of Physiol. Chem., Grad. School of Med., Univ. of Penna.). In a previous report an inverse correlation between total body water and body fat was recorded (Hardy, J. D., and Drabkin, D. L.: Am. J. Med. Sci., **219**, 108, 1950). The object of the present study was to determine the relation of plasma volume and sodium thiocyanate (SCN) space to total body water and body weight.

Eight healthy male adults were studied. Individuals of the same height and others of varying heights were selected to cover a wide range in body weight. Total body water was measured with D_2O (heavy water), "extracellular fluid" with sodium thiocyanate and plasma volume with the blue dye T-1824.

Plasma volume ranged from 2.75% to 5.65% of body weight, with a mean of 4.24% and an average percentage deviation of 22%. It ranged from 6.2 to 7.8% of total body water, with a mean of 6.96% and an average percentage deviation of 7%.

Thiocyanate space ranged from 30.4 to 37.6% of body water and from 15.7 to 22.8% of body weight. In the very lean individuals, the SCN space was fairly predictable and was usually 31 to 32% of body water and 21 to 22% of body weight. The SCN space represented a larger fraction of body water in the obese than in the very lean subjects. Possible explanations for this finding are suggested.

It is concluded that both plasma volume and SCN space are more closely correlated with total body water than with body weight. Since total body water is a function of the lean body mass, plasma volume and SCN space are also functions of the lean body mass. All 3 body fluid compartments bear an inverse relationship to body fat.

The Incorporation of Radioactive Orotic Acid into Pyrimidine Nucleotides of Nucleic Acid by Slices of Rat Liver. L. L. WEED, M.D., and D. WRIGHT WILSON, Ph.D. (Dept. of Physiol. Chem., School of Med., Univ. of Penna.). The *in vitro* incorporation of radioactive orotic acid into the pyrimidine nucleotides of nucleic acid has been studied with slices of rat liver and Walker rat carcinoma. Slices (6-8 gm.) were incubated for 4 hours in Krebs saline with phosphate buffer and 10 mg. of radioactive orotic acid. The nucleic acid was extracted from lipid-free trichloroacetic acid precipitate of homogenized slices by a 24-hour extraction with 10% sodium chloride. The nucleic acid was hydrolyzed in 1 N HCl giving a mixture of purine bases and the pyrimidine nucleotides, uridylic and cytidylic acids. These were separated chromatographically on paper and on resin columns. Eluates from paper containing a single constituent were run through the resin columns to assure purity, then quantitated spectrophotometrically, evaporated down on plates and the radioactivity determined with a windowless counter. In a single experiment with liver slices the actual counts in uridylic and cytidylic acids were 120 and 67 or 305 counts/min./mg. and 118 counts/min./mg. of pyrimidine base respectively. There was no detectable radioactivity in the guanine and in the 10 counts in .540 mg. of adenine. Many confirmatory experiments have been done. In tumor slices the counts per mg. of uridylic and cytidylic acids were 2590 and 542 respectively. Experiments similar to the above are being conducted using radioactive carboxymethylidene-hydantoin instead of radioactive orotic acid as a precursor.

Biosynthesis of Radioactive Fatty Acids and Cholesterol in Experimental Diabetes. ROSCOE O. BRADY, M.D., and SAMUEL GURIN, Ph.D. (Dept. of Physiol. Chem., School of Med., Univ. of Penna.). In an earlier report, the results of investigations upon the *in vitro* synthesis of radioactive fatty acids and cholesterol were described. It was found that liver slices from normal rats can readily convert labeled acetate, pyruvate, butyrate, hexanoate, and octanoate to long chain fatty acids and

cholesterol. These precursors are apparently first changed by liver slices to 2-carbon units which may subsequently be recondensed to form long chain fatty acids. Insulin was found to stimulate the incorporation of acetate into long chain fatty acids.

Liver slices from alloxanized rats behave in a decidedly different fashion. The incorporation of acetate and other substrates mentioned above into long chain fatty acids is drastically reduced to nearly 1/20 of that of normal. Fatty acid synthesis was not re-established by the addition of insulin, glucose, fructose-6-phosphate, oxalacetate, or α-keto glutarate to the incubating medium. However, this liver tissue which is apparently almost totally incapable of synthesizing long chain fatty acids from 2-carbon fragments continues to utilize the labeled substrates for cholesterol and acetoacetate formation. In addition to acetate and acetaldehyde, we have observed that acetoacetate is incorporated into cholesterol without prior fragmentation to a 2-carbon unit. The fact that acetoacetate is not utilized by liver slices for long chain fatty acid synthesis is further proof that acetoacetate is not broken down by rat liver to a 2-carbon fragment.

The above observations have been repeated and confirmed with liver slices from normal and pancreatectomized cats. Liver slices from Houssay cats retain a normal ability to synthesize fatty acids from acetate, and again insulin enhances the amount of substrate incorporated into the long chain fatty acids.

It would appear from these results that neither insulin nor the hormones of the pituitary are necessary for fat synthesis by liver. The pituitary, however, appears to secrete a principle which is capable of inhibiting the synthesis of long chain fatty acids by the liver. Insulin appears to counteract the inhibitory effect of the pituitary upon this process.

BINDERS AVAILABLE FOR YOUR COPY OF THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

In response to numerous requests, we can now offer subscribers attractive and sturdy binders large enough to hold six issues of The American Journal of Medical Sciences.

These binders will not only help to preserve your copies of The Journal, but will prove a definite asset to the appearance of your library. Binders are dark blue fabrikoid, with the name: AMERICAN JOURNAL OF THE MEDICAL SCIENCES, stamped in gold. Price, $2.00 each.

BOOK REVIEWS AND NOTICES

GERMAN AVIATION MEDICINE. WORLD WAR II. By 56 GERMAN AUTHORS. Prepared under the auspices of MAJOR GENERAL HARRY G. ARMSTRONG, Surgeon General, U. S. Air Force. In 2 vols. Pp. 1302; 920 figs., Washington, D.C.: U. S. Govt. Printing Office, 1950. Price, $8.50.

THIS is the result of the efforts of the U. S. Air Force to secure from German scientists authoritative information about their scientific accomplishments in the field of aviation medicine. Considering the confusion and destruction of facilities and documents that existed in Germany when the war ended, the result is a magnificent tribute both to the American military and civilian personnel engaged in the project and to the wholehearted cooperation of the Germans. The 2 volumes cover such topics as development and organization; aerodynamics and aviation medicine; physical and physiological fundamentals of high altitude; oxygen equipment; acceleration; vibration; problems of heat and cold; special senses; selection, training and care of the flier; emergency procedures; effects of air warfare on civilian population; blast effects.

The sections which the Reviewer feels competent to judge are compiled with characteristic German thoroughness, are for the most part sufficiently authoritative and complete to serve as reviews of the current status of these questions, and reveal a combination of fundamental theory with experimental resourcefulness and skill which in most instances were equal to, and in some were superior to, the corresponding activities in this country. Our complacency may be jolted by finding here descriptions of the multiple factor theory of respiratory control, the effects of hyperventilation on the cerebral circulation, and accounts of long experience with continuous electrical gas analyzers, oximeters, capacitance manometers, automatically operated pressure breathing regulators, warning devices activated by the electroencephalogram, successful training programs at high altitude stations, practicable devices for using liquid oxygen and chemical oxygen generators, and an account of the human centrifuge, dating to the first successful installation in the Charité Hospital in Berlin early in the 19th century. The German difficulties in securing definitive estimates of the value of different diets and drugs were identical with ours. Our aviation masks were better than theirs, and we surpassed them in developing effective body armor, antigravity suits, and practical equipment for using positive pressure breathing, which they dismissed as being too dangerous to the circulation and too uncomfortable. Their studies of extremely high altitudes, of the gravity-free state of flight in space, and of the effects of explosions fire, and dust on civilian populations contain many lessons for us. The translation from German to English is excellent, as is the paper. Some of the illustrations are rather poor, but considering that many were reproduced from the authors' memories this is not surprising.

These volumes deserve careful study by all physiologists, by physicians interested in aviation medicine or in the current preparations for civilian defense, by aviation engineers, and by lay writers seeking hitherto inaccessible information about the little-known problems of flight through the extremely high temperatures of the upper atmosphere and in the gravity-free state of interplanetary space. At their present price these volumes are a great bargain for any library. C. S.

MEDICAL PARASITOLOGY. By WILLIAM G. SAWITZ, M.D., Assoc. Prof. of Parasitology, Jefferson Medical College. Pp. 296; 90 figs., 1 color plate. Phila.: Blakiston, 1950. Price, $4.25.

THIS book, "intended as a lecture and laboratory guide for students in their course in Medical Parasitology", contains 5 sections: 1, Protozoa, 2, Helminths, 3, Arthropods, 4, Technics for Laboratory Diagnosis, and 5, Treatment. Dr. Sawitz has attempted to condense medical parasitology to essentials that should be included in the medical curriculum. The advantages of a condensation are obvious if, in the process, the material is presented so that the student obtains an adequate understanding of the subject. This Reviewer does not believe that such an understanding will be readily obtained from this book: diagnostic methods are inadequately described; geographic distribution of the parasites, their frequency in the population, and epidemiological factors are presented too briefly or ignored completely; many of the drawings will require considerable verbal explanation to be understood. The book cannot be recommended over others in this field. H.R.

THE ESOPHAGUS AND PHARYNX IN ACTION. By WILLIAM LERCHE, M.D., Formerly Assoc. Prof. of Surgery, Univ. of Minnesota. Pp. 229; 105 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $5.50.

THE initial purpose of the research de-

scribed in this book was to throw light on the closing mechanism at the cardia. When the constrictor cardiae was found in contraction in specimens that had remained in the state of regurgitation, the study consequently was extended to an investigation of the relation of structure to function in the stretch of alimentary canal from cardia to pharynx during acts of regurgitation and deglutition. The various theories pertaining to the closing mechanism at the cardia indicate that a number of problems involving anatomy and physiology are still unsettled. This book should prove of considerable interest to surgeons, pathologists and anatomists concerned with the relation of structure to normal and disturbed function of the esophagus and pharynx, and with the action of the upper esophagus and pharynx. The illustrations, paper and printing are excellent. N.F.

METHODS OF TISSUE CULTURE. By RAYMOND C. PARKER, Ph.D., Assoc. Prof. of Experimental Cytology, Univ. of Toronto. 2d ed. Pp. 294; 73 ills. New York: P.B. Hoeber, 1950. Price, $7.50.

THIS edition is really a brand new book, and an excellent one. All of the modern methods of tissue culture are carefully described, including chapters on preparation of various media and sterilization procedures. There is an excellent chapter on animal cell nutrition, and another on growth measurements and their interpretation. This book is not a dreary description of how to do something; it is fresh, stimulating and packed with interest for the biological investigator. It is without doubt the finest book of its kind available and it most certainly should be in every biologist's library. D.C.

DIAGNOSIS AND TREATMENT OF TUMORS OF THE HEAD AND NECK. By GRANT E. WARD, M.D., F.A.C.S., and JAMES W. HENDRICK, M.D., M.S., Univ. of Maryland and the Johns Hopkins Univ. Sch. of Med. Pp. 818; 637 ills. Balt.: Williams & Wilkins, 1950. Price, $15.00.

HERE is a complete text on the management of head and neck tumors based on an experience of over 20 years. Dr. Ward's training in electrosurgery and radio therapy as well as in general surgery has given him an approach available to few.

The long-term follow-up results and a close regard for the nature of the lesions, rather than the use of arbitrary standards, form the basis for the methods of treatment. This is well demonstrated in their short series of patients with cancer of the floor of the mouth and tongue treated by preöperative radiation and early surgical extirpation.

Ward and Hendrick advocate radical surgical extirpation, by electro-surgery or scalpel, for almost all advanced lesions. Every attempt is made to apply the principles of reconstructive surgery at the primary operation, or at subsequent ones, in an effort to restore function and improve appearance. They are well supported in this by other members of the respective hospital staffs who have contributed sections on plastic and prosthetic repair.

General surgeons, surgical specialists of the various components of the head and neck, and students will find this book useful. The references are well chosen. H.R.

A TEXTBOOK OF HISTOLOGY. By E. V. COWDRY, Prof. of Anatomy, Washington Univ. 4th ed. Pp. 640; 337 ills., 13 color plates. Phila.: Lea & Febiger, 1950. Price, $8.50.

THIS new edition presents numerous effective additions and revisions, and ranks as a leader for teaching or reference purposes. Correlation of structure and function is clearly presented. The numerous illustrations are exceptionally good. The bibliography has been extended considerably and a new section on historical aspects added. The inclusion of recent research developments also serves to render the text outstanding in the field of histology. I.R.Z.

TECHNIQUES IN BRITISH SURGERY. By RODNEY MAINGOT, F.R.C.S. Pp. 734; 473 ills. Phila. W. B. Saunders, 1950. Price, $15.00.

TWENTY-NINE leading surgeons have contributed 32 chapters on a variety of surgical problems to make up this book on British Surgical Techniques. Several fields of surgery (orthopedic, thoracic, urologic, neurosurgery) are included, although no attempt is made to cover any field in its entirety. The approach to surgical problems is practical, and technique at the operating table is stressed.

The surgical specialist in America will find this volume, based chiefly on British experience, to be provocative and stimulating. It is recommended more for him than for the undergraduate or young graduate student of surgery. W. F., Jr.

HANDBOOK OF MEDICAL PROTOZOOLOGY. By CECIL A. HOARE, D.Sc. (Lond.) Pp. 334; 43 ills., 3 in color. Balt.: Williams & Wilkins, 1950. Price, $7.00.

THE subject matter of this book is arranged in 3 sections: 1) a general account of the parasitic protozoa of man, their place in the animal kingdom, their structure, manner of

reproduction, physiological adaptations, classification and ecology; 2) a systematic account of these organisms in which morphology, life history, host-parasitic relationships, pathogenic activity, incidence, geographic distribution, epidemiology and diagnosis are presented in adequate detail; and 3) description and evaluation of laboratory methods of diagnosis.

This is a concise, up-to-date treatment of the subject which, to quote from the preface, "is intended primarily to fill a gap in the medical curriculum by providing students and practitioners of medicine with all essential information regarding protozoa living in man and the diseases caused by them." The book does this and more. H.R.

CLINICAL EXAMINATION OF PATIENTS. By JOHN FORBES, M.D., and W. N. MANN, M.D., Guy's Hospital, London. Pp. 323; 60 ills. Balt.: Williams & Wilkins, 1950. Price, $4.50.

THE Authors of this small volume, experienced teachers of physical diagnosis, have written this book primarily for the medical student; it presents to him clearly and logically the fundamentals of the clinical examination: history-taking and physical examination. The value and limitations of each portion of the clinical examination are demonstrated, and a modern and practical approach is outlined. The cherished signs of older clinicians are not retained for sentimental or other reasons where modern studies have shown them to be of limited value or based on misconception. While the book is not loaded with extensive considerations of laboratory findings, Roentgen-ray or electrocardiograph interpretations, reference is made to them to indicate wherein they are essential to supplement or corroborate clinical impressions. This publication will serve as an excellent text for classes in physical diagnosis. J. L.

RECENT ADVANCES IN PHARMACOLOGY. By J. M. ROBSON, M.D., Guy's Hosp., Univ. of London. Pp. 418; 46 ills. Phila.: Blakiston, 1950. Price, $5.50.

THIS is an extremely well written, documented and indexed digest of important recent developments in the pharmacology and clinical use of a number of drug groups. Discussion of drugs acting upon the autonomic nervous system, the myoneural junction and the hemopoietic system, as well as the antihistaminics, antibiotics and antimitotics is especially complete. Sections dealing with the mode of action and fate of drugs increase its value. C. L.

THE TRUTH ABOUT YOUR EYES. By DERRICK VAIL, M.D. Pp. 180. New York: Farrar, Straus, 1950. Price, $2.50.

THIS is a short and compact exposition of how the human eye works. Written for the lay public, it contains much useful information simply expressed. It would be a good book for ophthalmologists to have on their waiting-room table. It can be recommended to anyone wishing to know the fundamental facts of the functions of the eye and the diseases from which the eye suffers. F. A.

CYTOLOGY OF THE HUMAN VAGINA. By INES L. C. DE ALLENDE, M.D., and OSCAR ORIAS, M.D., Mercedes and Martin Ferreyra Inst. of Med. Invest., Córdoba, Argentina. Pp. 286; 75 ills., 15 in color. New York: P. B. Hoeber, 1950. Price, $7.50.

THIS book represents a comprehensive and careful study based upon the direct observation of a large amount of material by the Authors. Topics covered include the cytology of the vagina in normal cycles, alterations in hormonal dysfunctions, and alterations in hormonal therapy. The morphologic changes in the vaginal cells are clearly and carefully described and are well correlated with ovarian physiology. Numerous charts and graphs are employed to supplement the text. The cells are illustrated by excellent color photographs. Despite the fact of translation from the Spanish, the text is easily read. The book is highly recommended. A. R.

DISORDERS OF THE BLOOD. By SIR LIONEL E. H. WHITBY, Regius Prof. of Physic, Univ. of Cambridge, and C. J. C. Britton, M. D. (New Zealand). 6th ed. Pp. 759; 94 ills., 12 color plates. Phila.: Blakiston, 1950. Price, $8.00.

HIGHLY appreciated by hematologists the world over, this book presents all the good points of the better class of British scientific works. The material is comprehensive and accurate, selection and presentation admirable and with good documentation, the style clear and easy, the bookmaking excellent with eye-saving typography and illustrations that illustrate, a rich and accurate index, and typographical errors almost completely absent. I know of no successful medical textbook that better applies to its selections Alexander Pope's advice: "Be not the first by whom the new are tried, nor yet the last to lay the old aside."

The present edition, the second since the war, has a text increased by more than 10%, with extensive revision of the chapters on

"haemopoietic metabolism, coagulation, the haemolytic anaemias, leukaemia, haemagglutination, pigment metabolism and technique." It maintains the high standards of previous editions, which, in the Reviewer's opinion, makes it the best book on hematology in existence today in any language. E. K.

IMMORTAL MAGYAR. SEMMELWEIS, CONQUEROR OF CHILDBED FEVER. By FRANK G. SLAUGHTER, M.D. Pp. 211; 5 ills. New York: Henry Schuman, 1950. Price, $3.50.

ONE of the most poignant chapters in the history of medicine is the story of puerperal fever, with its terrific mortality brought about by the very people—doctors and nurses—whose lives were dedicated to saving life and relieving pain. In this story none played a more tragic role than Semmelweis who, having correctly discovered independently and announced the cause and a potent treatment for this plague, was so persecuted by misguided colleagues that first his professional career and then his sanity were wrecked. He soon after died at the early age of 45 of the same pyemia that had led him 18 years earlier to his famous discovery. Oliver Wendell Holmes, to be sure, had a few years before Semmelweis publicly maintained the contagiosity of the disease and had met from Hodge and Meigs, two of the leading obstetricians of this country, the same opposition that Semmelweis found in Europe. The latter did not live to see, as did Holmes, the general acceptance of their doctrines, which necessarily awaited a correct interpretation of the etiology of infection.

Dr. Slaughter, who retired from the practice of surgery several years ago into full-time authorship, is now better known in the latter capacity. This vivid story loses nothing in the telling. While emphasizing his indebtedness to other writers on the subject, he presents a very readable account of the life of Semmelweis. The fervid but long fruitless efforts to solve the problem of childbed fever, the discovery suggested by the autopsy on his colleague, Kolletchka, the triumph of reaction, the eventual breakdown of his sanity, all find their place in this instructive volume. E. K.

THE 1950 YEAR BOOK OF MEDICINE. Edited by PAUL B. BEESON, *et al.* Pp. 819; 136 ills. Chicago: Year Book Publishers, 1950. Price, $5.00

THIS is the 50th Anniversary Volume; and it contains 20% more material than previous editions. The editors have selected the articles for review with discrimination, as would be expected from the reputation of the editors in charge of the various sections. In addition to the customary abstracts, each editor has introduced a section with a progress review that covers the advances of the past 10 years. These reviews are fascinating and instructive. The Year Book of Medicine continues to perform its excellent service of abstracting the best in today's medical literature for the benefit of the general physician. J. L.

CANCER OF THE COLON AND RECTUM. By FRED RANKIN, M.D., Clinical Prof. of Surgery, Univ. of Louisville, and A. STEPHENS GRAHAM, M.D., Assoc. Prof. of Surgery, Medical Coll. of Virginia. 2d ed. Pp. 427; 148 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $7.50.

THIS work is indeed a valuable contribution to the field. The Authors discuss all phases of malignancy of the large bowel with the sure skill and knowledge that can come only from a rich clinical experience.

The sections describing symptoms and diagnosis, mortality and end-results, and preoperative and postoperative care are especially recommended. The sections presenting operative procedures receive less objective emphasis, but this may have been the Authors' intent. J. H.

THORACIC SURGERY. By RICHARD H. SWEET, M. D., Assoc. Clinical Prof. of Surgery, Harvard Univ. Pp. 345; 155 ills. Phila.: W. B. Saunders, 1950. Price, $10.00.

THIS practical volume is concerned primarily with operative technique in thoracic surgery. It is based on the concept "that any properly qualified surgeon can acquire with relative ease a satisfactory proficiency in thoracic surgery by employing the techniques herein described." In carrying out this objective the Author develops concisely and in well illustrated detail the surgical procedures which have proved valuable to him during his outstanding career in thoracic surgery.

The most complete coverage is given to pulmonary resection, while controversial subjects, experimental work, and operations of historical interest only are avoided. The volume should be of great value to the young student of thoracic surgery because of its fundamental nature, and of interest to all as an exposition of the techniques practiced in the Author's clinic. D. C.

THE CLINICAL USE OF RADIOACTIVE ISOTOPES. By BERTRAM V. A. LOW-BEER, M.D., Assoc. Prof. of Radiology, Univ. of California. Pp. 436; 105 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $5.00.

FROM the diversity of source material relative to medical applications of radioactive isotopes, there has been an urgent need for a coordinated reference and text. This volume, in large part, fulfills that need. It is well integrated and indexed as well as readable.

The physics section is comprehensive without being tedious or too involved in mathematics. The clinical material is well covered, and, although it seems to pass quickly between unrelated subjects, this is an accurate reflection of the real situation in this field.

The volume will soon be outdated—is already so in some clinical respects—but this must be the fate of all books on the applications of isotopes until greater stability is attained. In the meantime, it is gratefully welcomed as required reading for all radiologists, radiological physicists, and physiologically-minded physicians in nearly every field. It is an admirable text for graduate students in radiology. E. P.

ATLAS DER HAUT- UND GESCHLECHTSKRANKHEITEN. VON DR. WALTER FRIEBOES, Direktor der Universitats-Hautklink, Berlin, und DR. WALTHER SCHÖNFELD, Heidelberg. Pp. 266; 428 ills., most in color. Stuttgart: Georg Thieme, 1949 (imported by Grune & Stratton, 1950). Price, $15.90.

MOST dermatologists pride themselves on having some of the older atlases of skin diseases in their libraries. With the appearance of the work of Frieboes and Schoenfeld it is now possible for medical practitioners to have a ready source of excellent illustrations of the more common cutaneous diseases. Although the brief text is written in German, this is not as serious a drawback for general acceptance of this book by the medical public as it would be if the text were the chief feature of this presentation. Of the 428 illustrations only a few are ill-chosen; particularly in the section on syphilis. The mechanical aspects of this volume are excellently executed; the paper is of fine quality; and the color of the reproductions nearly perfect. The book is of convenient size for an atlas. A special feature is a number of dermatograms (impression prints) in black and white. This form of analysis ("finger" print) of certain dermatoses is worth further study. H. B.

SEROLOGY WITH LIPID ANTIGEN. By REUBEN L. KAHN, M.S., D.Sc., Assoc. Prof. of Dermatology and Syphilology, Univ. of Michigan. Pp. 327; 27 figs. Balt.: Williams & Wilkins, 1950. Price, $6.00.

THE text is divided into 4 parts: Introduction; Serology of Syphilis—with Kahn Procedures; Technique of Kahn Procedures; and Practical Aspects of Universal Reaction. At the end of each of the 17 chapters is a bibliography. Essentially, this new book is a summary of the observations and experimental work of the Author compiled during many years of laboratory investigation. Although the average serologist might prefer a text that includes analyses of other serology tests for syphilis, the Kahn test is so widely recognized that the laboratory worker should welcome the publication of a treatise upon it by the most eminent authority on the subject. The underlying mechanism and the general applicability of all the commonly employed tests for syphilis using lipid antigens, moreover, are adequately discussed. It is when the Author recounts and interprets the well documented experiments of the so-called "universal serologic reaction" as an explanation for "false positive" and "false negative" reactions for syphilis, however, that the thoughts of the reader are stimulated. R. N.

THE BIOLOGY OF HUMAN STARVATION. By ANCEL KEYS, AUSTIN HENSCHEL, OLAF MICKELSON, HENRY LONGSTREET TAYLOR, with ERNST SIMONSON, ANGIE STURGEON SKINNER, and SAMUEL M. WELLS. In 2 vols. Pp. 1385; 158 ills. Minneapolis: Univ. of Minn. Press, 1950. Price, $24.00.

PROFESSOR KEYS and his collaborators have assembled in this treatise the details of their investigations on starvation in human "guinea pigs". Their studies, which were carried out on conscientious objectors during the recent war, have already received much publicity in the popular press. The publication of the details of the experiments has accordingly been awaited by all those who are concerned with the problems of semi-starvation and of rehabilitation. The historical aspects of the subject are also covered in the text.

Morphologists, biochemists, physiologists, and psychologists as well as physicians will have occasion to refer frequently to this monumental work. It may safely be assumed that these volumes will soon take their place among the classic reference works on nutrition. M. C.

POST-GRADUATE LECTURES ON ORTHOPEDIC DIAGNOSIS AND INDICATIONS. Vol. 1. By ARTHUR STEINDLER, M.D., Prof. of Orthop. Surg., State Univ. of Iowa. Pp. 302; 400 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $7.50.

THIS, the first of a 4 volume series covers in two parts, Propedeutics in Orthopedic Diagnosis, and Congenital Deformities and Disabilities. The material, arranged in lecture form, represents a condensation of lectures given by the Author to graduate students. The presentation is well organized and pointed throughout without being dogmatic. Historical orientation in each subject is particularly good and the bibliography at the end of each section, although not intended to be complete, is adequate.

The principles of treatment are presented broadly, which would seem to make the volume particularly valuable to the younger student who requires a well organized background for further study in controversial details. There is little with which to disagree in any of the presentations, though some readers may question the classification of congenital clubfoot and flatfoot. All congenital deformities are discussed, particularly clubfoot, dislocation of the hip, and disabilities of the spine. Achondroplasia, Ollier's Disease, Multiple Exostoses, Morquio's Disease, and Fragilitas Ossium are placed among the congenital disabilities.

This volume is recommended for the library of any serious student of Orthopedic Surgery. For the younger student, it will serve as an introduction and starting point; for the older, as a quick reference for proper classification of these often confusing and sometimes rare conditions. E. R.

RESEARCHES IN BINOCULAR VISION. By KENNETH N. OGLE, Ph.D., Mayo Clinic. Pp. 345; 182 figs. Phila.: W. B. Saunders, 1950. Price, $7.50.

THIS book is the outcome of the Author's investigations in the Dartmouth Eye Institute. It is a well organized and authoritative treatise on binocular vision but is intended only for those who are well acquainted with the subject from the theoretical aspect.

Part I deals with the organization and sensory cooperation of the two retinas. Part II, with the fusional processes in binocular single vision. Part III concerns the problems in binocular vision when changes are made in the relative magnification of the images of the two eyes, and this naturally leads into Part IV, which is the experimental and theoretical bases for aniseikonia.

The book will undoubtedly be received with acclaim by experts in this field. F. A.

NEW BOOKS

Aetios of Amida, The Gynaecology and Obstetrics of the VIth Century, A.D. Translated from the Latin Edition of Cornarius, 1542, and Annotated by JAMES V. RICCI, M.D., Clinical Prof. of Gynaecology and Obstetrics, New York Med. Coll. Pp. 215. Phila.: Blakiston, 1950. Price, $7.00.

THIS compilation by the last of the great writers of the Greco-Roman period constitutes the 16th book of his voluminous Encyclopedia. A good picture of gynecological and obstetrical knowledge in the 6th century, it has been translated from the best Latin translation (1542) by one who has first hand knowledge of his field and has written copiously about its historical aspects. This, apparently the 1st English translation, has been augmented by notes that occupy more than half the volume and add considerably to its interest.

E.K.

The Biochemistry of B Vitamins. By ROGER J. WILLIAMS, ROBERT E. EAKIN, ERNEST BEERSTECHER, JR., and WILLIAM SHIVE, Dept. of Chemistry, Univ. of Texas. Pp. 750. New York: Reinhold, 1950. Price, $10.00.

IN this monograph each author has written one of the following sections: characterization, distribution, assay and biogenesis of B vitamins; the role of the B vitamins in animal and plant organisms; the comparative biological activities of the B vitamins in animal and plant organisms; the comparative biological activities of the B vitamins and related compounds.

Besides giving a well digested and critical survey of each of these fields, they have developed their individual viewpoints upon the adequacy and the interpretation of current knowledge. The material presented is of vital interest to workers in many fields; this volume is recommended as the most up-to-date evaluation of this subject available. H.V.

Introduction to the Regulation of Blood Pressure and Heart Rate. By CORNEILLE HEYMANS, M.D., Prof. of Pharmacology, Univ. of Ghent. American Lect. Ser. 43. Pp. 60; 25 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $2.00.

A concise presentation of the mechanisms concerned in blood pressure homeostasis by one who has contributed considerably to the experimental study of the subject.

Coagulation, Thrombosis, and Dicumarol. By SHEPARD SHAPIRO, M.D., Ass't Prof. of Clinical Medicine, and MURRAY WEINER, M.D., Fellow in Medicine, New York Univ. Pp. 131; 7 figs. New York: Brooklyn Medical Press, 1950. Price, $5.50.

Essay on the Cerebral Cortex. By GERHARDT VON BONIN, M.D., Prof. of Anatomy, Univ. of Illinois. Pp. 168; 83 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $3.75.

The Place of Statistical Methods in Biological and Chemical Experimentation. By EDWIN J. DE BEER, *et al.* Ann. N. Y. Acad. Sci., 52, 789-942, 1950; illustrated. Price, $2.25.

Die Fokale Erkrankung des Körpers. VON DR. MED. S. G. FUDULLA. Pp. 172. Stuttgart, Germany: Marquardt & Cie., 1950. Price, DM 8.50.

Aktive Bewegungstherapie bei Inneren Erkrankugen. VON R. TRUMPP. Pp. 148; illustrated. Stuttgart, Germany: Marquardt & Cie., 1950. Price, DM 9.50.

Radiation Therapy in the Management of Cancer of the Uterine Cervix. By SIMEON T. CANTRIL, M.D. American Lect. Ser. 55. Pp. 200; 86 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $5.00.

Medical Mycology. By FREDERICK REISS, *et al.* Ann. N. Y. Acad. Sci., 50, 1209-1404. Illustrated. New York: The Academy, 1950. Price, $2.75.

Terramycin. By C. S. KEEFER, J. D. ADCOCK, *et al.* Ann N. Y. Acad. Sci., 53, 221-460. Illustrated. New York: The Academy, 1950. Price, $3.00.

The Physician Examines the Bible. By C. RAIMER SMITH, M.D. Pp. 394. New York: Philosophical Library, 1950. Price, $4.25.

MEDICAL subjects in the Old and New Testaments and Apocrypha are examined as to etiology, diagnosis and prognosis, and compared with present day practices.

Principles of Ophthalmology. By THOMSON HENDERSON, M.D., Senior Surgeon, Nottingham and Midland Eye Infirmary, England. Pp. 230; 63 ills. New York: Grune & Stratton, 1950. Price, $4.50.

Plasma Clot Suture of Peripheral Nerves and Nerve Roots. By I. M. TARLOV, M.D., Assoc. Prof. of Neurosurgery, N. Y. Med. Coll. Pp. 116; 47 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $5.50.

Acta Argentina de Fisiologia y Fisiopatologia. Vol. 1, No. 1, Pp. 291. Cordoba, Argentina: Univ. Nac. de Cordoba, 1950. Price not given.

WE are glad to announce the appearance of the first number of this new Argentinian journal.

Biological Oxidation of Aromatic Rings. Edited by R. T. WILLIAMS. Biochem. Soc. Symposia 5. Pp. 96. New York: Cambridge Univ. Press, 1950. Price, $2.00.

Your Pregnancy. By ANNA ROOSEVELT and LEO DOYLE, M. D. Pp. 178. New York: Henry Holt, 1950. Price, $2.50.

For the general public. Contains the usual information and advice.

La Dissociation Auriculo-ventriculaire dans l'Infarctus du Myocarde. Par DR. ROGER VIALARD. Pp. 198; 19 figs. Paris: Libr. Arnette, 1950. Price not given.

Rh Organization and Research in the Netherlands. Introduced by DR. A. B. F. A. PONDMAN, Prof. in Bacteriology and Serology, Univ. of Groningen. Pp. 185. Leiden: H. E. Stenfert Kroese's Uitgev.-Mij N. V., 1950. Price, 5 fl.

Schizophrenic Art: Its Meaning in Psychotherapy. By MARGARET NAUMBURG. Preface by THOMAS A. C. RENNIE, M.D. Pp. 246; 63 figs. New York: Grune & Stratton, 1950. Price, $10.00.

Carbon Dioxide Therapy. A Neurophysiological Treatment of Nervous Disorders. By L. J. MEDUNA, M.D., Prof. of Psychiatry, Univ. of Illinois. Pp. 248; 8 ills. Springfield, Ill.: Charles C Thomas, 1950. Price, $5.00.

NEW EDITIONS

THE PATHOLOGY OF INTERNAL DISEASES. By WILLIAM BOYD, M.D., Prof. of Pathology and Bacteriology, Univ. of Toronto. 5th ed. Pp. 866; 391 ills., 11 color plates. Phila.: Lea & Febiger, 1950. Price, $11.00.

THIS popular text has been brought up to date without change in format. Intended primarily for the internist and student, it is written from the point of view of special pathology. The various organs and their diseases are dealt with in order. A clear style, good organization of material and frequent correlation with clinical findings should make the book of special value to its intended audience. C.B.

The Management of Obstetric Difficulties. By PAUL TITUS, M.D., St. Margaret Memorial Hospital, Pittsburgh. 4th ed. Pp. 1046; 446 ills., 9 color plates. St. Louis: C. V. Mosby, 1950. Price, $14.00.

THE basic plan of this book remains unaltered, but the material has been revised to include new knowledge and technique. The bibliography following each chapter has been brought up to date. A most commendable effort has been made to attain greater uniformity in certain terms and classifications. The book can be recommended as a useful reference and guide to physicians attending obstetric patients. P. K.

Index photographed at the
beginning for the convenience
of the microfilm user.

More Complete Therapy for Cough Control*

Pyribenzamine® Expectorant

A Unique Combination of Non-narcotic Drugs

Anesthetic
Decongestant
Antispasmodic
Antihistaminic

FORMULA—Each teaspoonful (4 cc.) contains 30 mg. Pyribenzamine (tripelennamine) citrate, 10 mg. ephedrine sulfate, and 80 mg. ammonium chloride.

DOSAGE—Adults, 1 to 2 teaspoonfuls every 3 to 4 hours. Children, half the amount at same interval. Followed by full glass of water.

PYRIBENZAMINE
A NO. 1 ANTIHISTAMINIC

Ciba PHARMACEUTICAL PRODUCTS, INC., SUMMIT, NEW JERSEY

2/1555M

release the FLOOD GATES

in Cardiac Edema Control

". . . the diuretic drugs not only promote fluid loss but in many instances also effectively relieve dyspnea . . . not only may the load on the heart be decreased but there may also occur an increase in the organ's ability to curry its load . . . With good average response the patient perhaps voids about 2000 cc. of urine daily, but in exceptional instances the amount rises to as high as 8000 cc."[1]

"Not only are the diuretics of immense value in cases of left ventricular failure . . . but where edema is marked, as it is most likely to be in failures occurring in individuals with chronic nonvalvular disease with or without hypertension and arrhythmia, their employment is often productive of an excellent response. In [edematous patients with] active rheumatic carditis (rheumatic fever) the use of these drugs may be life-saving."[2]

Salyrgan-Theophylline is effective by muscle, vein or mouth.

salyrgan®-THEOPHYLLINE

BRAND OF MERSALYL AND THEOPHYLLINE

TIME TESTED • WELL TOLERATED

AMPINS (1cc and 2cc) • AMPINS (1cc) • TABLETS

Winthrop-Stearns INC.
NEW YORK, N.Y. WINDSOR, ONT.

1. Beckman, H.: Treatment in General Practice. Philadelphia, Saunders, 5th ed., 1946, 704-705.
2. Beckman, H.: Treatment in General Practice. Philadelphia, Saunders, 6th ed., 1948, 744.

Salyrgan, trademark reg. U. S. & Canada—Ampins, reg. trademark of Strong Cobb & Co., Inc.